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Role of hydrocarbon pollutants, salinity, tidal height, bioenergetics and competition in colonization of oyster reefs by commensal assemblages

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ROLE OF HYDROCARBON POLLUTANTS, SALINITY, TIDAL HEIGHT, BIOENERGETICS AND COMPETITION IN COLONIZATION OF OYSTER REEFS BY COMMENSAL ASSEMBLAGES

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Abstract

Effects of hydrocarbons, salinity, tidal height, bioenergetics and competition on the oyster reef fauna were studied. Dried oyster shell was exposed to crude oil in the laboratory and plastic trays filled with control and oil-exposed shell were then deployed at two locations, in two seasons and at two tidal levels, with immersion periods of two and five weeks. Salinity exhibited a significant effect with sub tidal site having higher diversity. Hydrocarbon effects were less prominent. To determine effects of cleaners, Corexit 9580 was applied both alone and in combination with oil on cultch. At high concentrations, the cleaner ameliorated hydrocarbon effects. Hydrocarbon effects seemed to be less prominent than salinity and aerial exposure.

In the next chapter, effects of salinity on the survival and bioenergetics of mud crabs *Panopeus simpsoni* and *Eurypanopeus depressus* were examined. Crabs were exposed to a range of salinities to determine the effects of salinity on tolerance. *P. simpsoni* exhibited a 28d LC_{50} of 6.97 PSU while *E. depressus* had a 28d LC_{50} of 0.19 PSU. Crabs were exposed to four salinities for bioenergetic measurements. Energy expenditure was highest at the lowest salinity and decreased as salinity increased. Scope for growth declined below 17.5 PSU. *E. depressus* was capable of surviving lower salinities than *P. simpsoni.* However, the physiological responses do not significantly differ between the two species.

In the next chapter laboratory experiments were conducted to see if these two crab species differ in resource holding potential. Crabs were placed in aquaria at two salinities and refugia were checked daily for shelter occupancy. To determine if resource

holding potential for refugia influenced predation risk, a second laboratory experiment was performed with a blue crab predator in each tank. The numbers of mud crabs of each species surviving were recorded. *E. depressus* was dominant over *P. simpsoni* in occupying the shelters at both salinity levels. *E. depressus* exhibited a higher survival in the presence of the predator. *E. depressus*'s ability to tolerate lower salinities, and its dominance in resource holding potential, may lessen predation risk and allow colonization of more estuarine sites.

Chapter 1
General Introduction

The eastern oyster (*Crassostrea virginica*) has long been recognized as an important species for its fishery value and the ecological services oysters provide (Lenihan and Peterson 1998). Oyster reefs provide a habitat for a variety of organisms, and they have been compared to coral reefs in terms of their structural heterogeneity and vertical relief (Harding and Mann, 1999). Although 70% of the organic matter filtered by oysters is assimilated (Newell, 1988), the remainder is available as a food source for benthic organisms. Oysters also act as ecosystem engineers (*sensu* Jones et al. 1994), creating extensive biogenic reefs. The secondary production of oysters and the complex three-dimensional reef structure attract numerous invertebrates and fishes (Harding and Mann 1999; Kennedy, 1996; Posey et al. 1999).

However, oyster reefs and estuarine salt marshes are perhaps the most vulnerable marine habitats to hydrocarbon pollution. Their low levels of tidal flushing make them vulnerable to oil spills (Grundlach and Hayes, 1978). Toxicological effects of petroleum hydrocarbons on marine invertebrates and fish are well documented (Neff and Anderson, 1981; Peterson, 2001). Neff (1985) summarized the effects of polycyclic aromatic hydrocarbons (PAH's), that can alter metabolism, cause tumor growth and disrupt enzyme function (Albers, 1992). Oil exposure also shifts the relative abundance of microbial flora, and impacts fish and invertebrate mortality, growth and reproduction (Rand et al. 1995). Considerable work has been done on the effects of hydrocarbon pollutants on coastal marsh vegetation (reviewed by Pezeshki et al. 2000). Although the

effects of hydrocarbons have been studied on oysters, the responses of invertebrates and fish that colonize oyster reefs are poorly studied.

In the second chapter, I studied the relative affects of hydrocarbons on oyster reef invertebrate and fish populations. I also examined how salinity, tidal height and season impact the colonization of oyster reefs. Trays filled with oyster shell were used as artificial oyster reefs. Results of these experiments indicated that hydrocarbon effects were minor compared with salinity and aerial exposure. The first chapter was submitted to *Marine Environmental Research*.

Estuarine organisms are exposed to fluctuating environmental factor gradients such as salinity and aerial exposure. Salinity is the most important environmental factor affecting distribution within estuaries, and certainly one of the most intensely studied factors. Two mud crab species of the family Xanthidae, *Panopeus simpsoni* (H. Milne Edwards) and *Eurypanopeus depressus* (Smith), are commonly found associated with oyster reefs in Gulf of Mexico. Little is known of what factors determine their distributions. The results of my second chapter indicated that hydrocarbon contaminants had little effect on mud crab distributions. *E. depressus* is more common in upper, more estuarine regions than *P. herbstii* in Alabama bays (May, 1974). Similar observations were made in the experiments conducted in the second chapter, and in the third chapter I therefore systematically studied the role of salinity in the bioenergetics of those two mud crabs. I investigated the effect of salinity on scope for growth as an indicator of sub-lethal stress. Comparisons of osmoregulatory ability were also made in an effort to explain the distribution of these two species. Although *E. depressus* survives at lower

salinities than *P. simpsoni*, both species had similar changes in scope for growth across a salinity range of 17-25 PSU, and their osmoregulatory ability did not differ.

Since these physiological responses did not differ and could not explain their distribution, I decided to examine the role of competition and predation. The fourth chapter documents how these factors play a role in determining the distribution of these two species. Availability of refuges influences the density and size structure of many marine crustacean populations (Steger, 1987; Caddy and Stamatopoulas, 1990; Beck, 1997). Brown et al. (2005) found that *E. depressus* were dominant over *P. simpsoni* for both food and shelter resources, and that the dominance hierarchy predicted resource-holding potential (RHP). I determined the ability of each species to successfully defend shelters in short supply in laboratory experiments at two salinities. *Callinectes sapidus* is capable of preying upon a variety of marine organisms including *P. simpsoni* and *E. depressus* (Seed, 1993). The survival of each species in the presence of a blue crab was examined to investigate relationships of shelter use, salinity and predation risk. I hypothesized that *E. depressus* would have higher survivorship because of its greater RHP. The results are discussed in relation to the distribution of these two mud crab species. Chapters three and four will be submitted to *Marine Biology*.

Chapter 2
Relative Importance of Hydrocarbon Pollutants, Salinity and Tidal Height in Colonization of Oyster Reefs by Commensal Assemblages

Introduction

Estuarine oyster reefs provide a solid substrate for colonization, when surrounding bare sediments are unsuitable for many species, and provide food, resources and protection from predators. Oyster reefs thus have a diverse fauna in comparison to surrounding mudflats (Bahr and Lanier, 1981), and a fauna that differs from species in *Spartina* stands (Zimmerman et al., 1989). Oyster reefs provide an important nursery habitat for many invertebrates and fish (Soniat et al., 2004). Vertical habitat complexity also determines the size and abundance of transient fish like sea bass, groupers and snappers (Harding and Mann, 1999; Lehnert and Allen, 2002). Resident species include invertebrates like penaeid shrimp, blue crabs, stone crabs, and larvae of commercially important fish like mangrove snappers (Bahr and Lanier, 1981). However, the nursery role of oyster reefs has been relatively unappreciated until recently (Beck et al., 2001).

Oyster reefs occur in the inter-tidal zone of coastal marshes along the Gulf of Mexico, because predators like oyster drills, stone crabs and black drum remove most sub-tidal oysters (Brown and Stickle, 2002). Inter-tidal sites are thus important refugia for bio-diversity, but understanding interactions in sub-tidal sites is also important, as oyster lease holders deploy “seed” oysters (small oysters) to sub-tidal sites. In general, oyster recruitment and adult biomass are highest in an intermediate salinity band of 5 – 15 PSU (Melancon et al., 1998). Lower salinities depress oyster reproductive success (Chatry et al., 1983) while losses to predation increase at coastal sites with higher salinities (Brown and Stickle 2002; Soniat et al., 2004). In particular, little is known about the ecology of the invertebrates and larval fish that use oyster reef structure as refuge from predators.

This study documents the relative importance of hydrocarbon pollutants, salinity, tidal height and seasonal changes to these invertebrates and fish.

Petroleum hydrocarbons have detrimental effects on many marine invertebrates and fish (Neff and Anderson, 1981; Suchanek, 1993; Peterson, 2001). Polycyclic aromatic hydrocarbons (PAH's) in particular have carcinogenic and mutagenic actions (Neff, 1985). Oysters bio-accumulate PAH's in lipids used for reproduction (Jackson et al., 1994), and, unlike some arthropods, cannot enzymatically degrade hydrocarbons (Neff, 1985). Hydrocarbons can also alter recruitment patterns of oysters, barnacles and bryozoans (Smith and Hackney, 1989; Levings and Garrity, 1992; McCoy and Brown, 1998; Banks and Brown, 2002).

Considerable work has been done on the effects of hydrocarbon pollutants on coastal marsh vegetation (reviewed by Pezeshki et al., 2000). Heavier molecular weight hydrocarbons can coat plant surfaces and interfere with photosynthesis, while lighter hydrocarbons actually penetrate and damage plant tissue. In comparison, less is known about the effects of hydrocarbons on invertebrates and fish that rely on oyster reefs for shelter. A few studies have however investigated the effects of cleaners or dispersants, that are often applied to disperse oil spills, on benthic macrofauna in general in marine systems (Griffiths et al., 1980; Chan and Chiu, 1985).

To determine the relative affects of hydrocarbons on oyster reef invertebrate and fish populations, I conducted the following field experiments. Plastic trays filled with dried oyster shell that had been exposed to hydrocarbons in the laboratory were used as "artificial" reefs to measure the effects of hydrocarbons on the reef community, along

with comparing these effects to spatial and temporal variation in the colonization rates of benthic macrofauna. This technique not only allowed replication of my treatments, but also minimized any impact on surrounding oyster reefs. My initial hypotheses were that the diversity and abundance of organisms on my artificial reefs would be lowered with the application of hydrocarbons. In addition, I hypothesized that diversity and abundance would be lowered at more estuarine sites (compared with coastal sites) because of the reduced salinity, and would be lowered at inter-tidal sites (compared with sub-tidal sites) because of increased aerial exposure. Finally, I hypothesized that invertebrate and fish diversity and abundance would be lower in experiments conducted in the winter, because of lower colonization rates in general at lower temperatures.

Materials and Methods

Basic technique

Oyster reefs are difficult to sample with traditional technologies like trawls, dredges, and seines because of their complex topology. Oysters grow in dense aggregations and have sharp shell edges. My sampling technique, using trays filled with oyster shell as artificial oyster reefs, is easy to deploy and replicate and gives quantitative data on species richness and abundances. Trays were 0.67 m x 0.67 m x 10 cm high impact-resistant plastic with a coarse mesh of approximately 3 cm. They were lined with 2 mm Vexar® mesh attached tightly to the inside of the tray frame with plastic cable ties. Each tray received approximately 8 L by volume of dried oyster shell. Oyster shells were intact, except shells were dis-articulated at the umbo. Oyster shells were roughly

10 – 15 cm in length, and were collected from seafood processors that had removed the meat. Each tray had two 1 meter ropes connecting opposite corners that were attached to a 1 meter long rope attached to a float. This rope bridle allowed the tray to be carefully lowered to the bottom and retrieved without spilling oyster shell.

When trays were retrieved they were immediately placed in large plastic tubs to capture any small (e.g. < 2 mm) invertebrates. Oyster shells were then individually washed in 5 gallon buckets and the water in the buckets and tubs was washed through a 1 mm mesh sieve to retain all invertebrates and fish > 1 mm. Organisms were immediately fixed in 10% formalin and later transferred to 80% ethanol in the laboratory. All fish and invertebrates were observed under a dissecting scope at 10 – 30 times magnification, and identified to genus and species using taxonomic keys (Gosner, 1971; Felder, 1973; Hopkins et al., 1989).

Experiment 1--Hydrocarbons and site differences

In 2001, I immersed cultch in Louisiana "sweet" (e.g., low sulfur content) crude oil for one week in the laboratory. Oyster shells were placed in 1 L glass beakers and oil was added to the beakers, which were then sealed with parafilm. After one week, shell was carefully removed from beakers and placed in the trays, which were enclosed in large plastic bags. Trays were immediately transported to field sites, and deployed in 1 meter of water. I compared colonization of trays with oil-soaked cultch to trays with control oyster shell after four weeks at a coastal site, Bayou Fourchon, near the Louisiana Universities Marine Consortium (LUMCON) laboratory and at an estuarine site 10 km up

the bayou, Leeville. Trays were deployed on a shallow, sub-tidal mud-sand beach at Bayou Fourchon, and at a similar depth and substratum type at Leeville. Bayou Fourchon had an average salinity of 22 ± 1.5 PSU, while Leeville averaged 10.2 ± 1.5 PSU during the experiment. Trays were deployed on the 11th of July 2001 and were picked up on the 8th of August 2001. There were five replicate trays for each treatment at each site. I conducted 2-way analyses of variance to compare species richness and the abundance of each invertebrate and fish species between the two sites and two hydrocarbon treatments. A 2-way analysis of variance was also conducted on the total number of organisms per tray, and a log transformation was performed on the latter as the data were not normally distributed according to the Shapiro-Wilks test.

Experiment 2--Interactive effects of oil and cleaner treatments

In spring 2002, I conducted a second experiment to explore interactive effects of hydrocarbons and cleaners. I considered it important to look at interactive effects because dispersants and cleaners can have worse effects when used in oil spill cleanups than the hydrocarbons alone (Griffiths et al., 1980; Chan and Chiu, 1985). Cultch was immersed in Louisiana sweet crude in the laboratory as above, and a solution of a commercially-available cleaner, COREXIT 9580, was applied as a spray when trays were deployed. The trays were deployed at the original site in Bayou Fourchon on the 20th of April, and were retrieved on the 26th of May 2002. The cleaner doses, 0.2 L/m^2 and 0.8 L/m^2, were based on Delaune et al., (1996), who used a dose of 0.2 L/m^2 to remove oil from marsh vegetation. There were three replicate trays for each treatment. Trays were

processed, and invertebrates and fish collected and counted, using the same methods as in the first experiment. The experimental design was a 2-way analysis of variance (presence or absence of oil vs. three levels of cleaners). The dependent variables were again the diversity and total abundance of organisms per tray.

Experiment 3--Effects of heavy crude oil, tidal height and colonization period

In fall 2002, cultch was exposed to heavier (e.g., denser) Venezuelan crude oil, using the same methods in the laboratory, and trays were deployed at an inter-tidal (± 0.5 m above MLW) and a sub-tidal (1 m below MLW) location at the same Bayou Fourchon site. Venuzeulan crude was used because its heavier specific gravity causes it to precipitate on reefs in an actual spill, unlike the lighter Louisiana crude that could float on water and disperse before coating oysters. Six replicate trays for each treatment were deployed between the 15th of July and the 23rd of July 2002. To determine if colonization period interacted with hydrocarbon contamination, half of the trays were retrieved after 2 weeks and the remaining trays were retrieved after 5 weeks. These colonization periods were chosen based on the time periods predicted in the literature for effects of hydrocarbon contaminants to be manifested for different groups. Effects on fish (either mortality or declines in abundance caused by emigration) may occur within a few days, while toxic effects on invertebrates may take as long as a month (Moles, 1998). The experimental design was a 3-way factorial arrangement of treatments (presence or absence of oil X two tidal heights X two colonization periods).

Experiment 4 - Seasonal effects

In winter 2003, cultch was pre-exposed to Venezuelan crude oil and cultch-filled trays were deployed at the same inter-tidal and sub-tidal sites at Bayou Fourchon on the 6th of January, and retrieved on the 8th of February, 2003. Trays were processed with the same methods, and these data were compared with the 2001 summer data set from the same sites to look at seasonal effects on invertebrate and fish species richness and total abundances. The logic behind this experimental contrast was that contaminant effects might be more severe in summer because of increased macrofaunal abundance and activity patterns. For example, oil spills during winter months have less serious effects on coastal macrophytes (Pezeshki et al., 2000). The experimental design was a 3-way factorial ANOVA (presence or absence of oil X two tidal heights X two seasons) with five replicates per treatment.

Gas chromatography/ mass spectrometry analysis

To determine how much oil actually remained on oyster shell after one month immersion, in summer 2004, I immersed cultch in Venezuelan crude oil for one week in the laboratory, as in earlier experiments. After one week, both oil-coated shells and control shells were subjected to Gas Chromatography/ Mass Spectrometry (GC/MS). Additional shells were then carefully removed from beakers and placed in trays that were enclosed in large plastic bags. On the 27th of May, trays were transported to the same coastal inter-tidal, coastal sub-tidal and estuarine site used earlier and deployed with the same methods in 1 meter of water. The trays were retrieved after one month, and the shell was placed in glass beakers sealed with parafilm and transported to the laboratory.

GC/MS readings were again taken for shell with mud and shell with mud removed. The logic was that animals colonizing trays would be exposed to a combination of oiled shell and overlaying, un-oiled sediment. Shell extractions were modified from the EPA SW-846 ultrasonic extraction method 3550B (US EPA, 1997). Initial oiled shell was placed in a pre-weighed beaker and weighed. Shell was then covered with dichloromethane (DCM) and spiked with surrogate standards (5-alpha-androstane for alkanes and phenanthrene-d10 for aromatics) to determine the extraction efficiency. The beaker was then placed in an ultrasonic bath for 15 minutes and the liquid layer was poured through a funnel lined with Whatman #2 150 mm filter paper containing anhydrous sodium sulfate into a round bottom flask. The extraction was repeated twice and the shell discarded. The extracts were rotary evaporated to 1 ml and pipetted into 4 ml glass vials with screw top caps. If more than 1 ml of sample remained, it was blown down to 1 ml with nitrogen gas (95% purity). The samples were wrapped with Teflon seal tape and refrigerated until analysis. For shells retrieved from the field, mud was scraped off one sample, and the shell was then extracted using the same method as initial shells. Another extraction was performed for shell with mud using the same methods. Finally, a reference oil extract was prepared by weighing 0.5 g of oil and adding 20 ml of DCM. The sample was then extracted using the same methods.

The samples were analyzed using a Gas Chromatograph (HP model # 5890A) coupled with a Mass Selective Detector (HP 5971 Series). Prior to using the GC/MS, a standard autotune was performed to ensure that the instrument was functioning according to operating protocols. For the petroleum hydrocarbons analyzed, the GC oven

temperature was programmed to be held at 55 ^{0}C for 3 minutes, and then increased to 280^{0}C at the rate of 5 ^{0}C/min, and finally increased to 300 ^{0}C at 0.50 ^{0}C/min. The injector and MS interface temperature for both GC/MS methods was set at 250 and 280 ^{0}C, respectively. A solvent blank and one of the calibration standards were placed at the beginning of the sequence. Data were obtained with a HP ChemStation macro. Analyte responses were transferred to a spreadsheet and the concentrations were calculated.

Results

Site and hydrocarbon differences

The community of invertebrates and fish using the artificial reefs for shelter was quite diverse (Table 2.1). Of the 10 species of crustacean arthropods, snapping shrimp and mud crabs were quite abundant, and blue crabs, grass shrimp and porcelain crabs were commonly collected. Hermit crabs, stone crabs and penaeid shrimp were rarely collected, and then only at the coastal site. The six species of fish were dominated in abundance by naked gobies. Gastropods were much more common at the coastal site, and were dominated in numbers by the small detritivorous mud snail, *Nassarius*. Based on both their lack of enzymatic systems to degrade hydrocarbons (see introduction) and their relative immobility, I would predict that the molluscs would be most susceptible to hydrocarbon contaminants.

There was an obvious effect of salinity on the diversity of invertebrates and fish (Fig. 2.1) with species richness halved at the more estuarine site ($F_{SITE} = 129$, $P < 0.01$). In comparison, hydrocarbons only reduced richness on the average from 11 to 9 species

at the estuarine site, and from 21 to 18 species at the coastal site, although the effect was still statistically significant ($F_{OIL} = 8.7$, $P = 0.01$). The results of individual analyses of variance indicated eleven of the species had higher abundances at the coastal site, versus only two with higher abundances at the estuarine site. In contrast, individual analyses of variance indicated only two species (the toad fish *Opsanus beta* and mud snail *Nassarius acutus*) had reduced abundances in oil-treated cultch. The mud snail, a small detritivore abundant at the coastal site, was however completely eliminated by the hydrocarbon treatment.

When the total number of organisms (both invertebrates and fish) collected per tray was analyzed, only the site effect was significant ($F_{SITE} = 27.6$, $P < 0.01$). The coastal site again had a higher abundance of organisms than the estuarine site, and mean abundances were reduced by 63 % at the estuarine site (Fig. 2.2). Although abundances were consistently depressed in oil treated cultch at both sites, the effect was only marginally significant ($F_{OIL} = 4.1$, $P = 0.06$), averaging 25 % over both sites.

Interactive effects of oil and cleaner treatments

The main effect of oil ($F_{OIL} = 4.8$, $P = 0.05$) was significant on diversity as was the interaction of oil and cleaner ($F_{INT} = 4.9$, $P = 0.05$) but not the cleaner main effect ($F = 1.0$, $P = 0.38$). Closer inspection of the richness values revealed little difference in diversity between oil- treated and control cultch in the absence of, or at low concentration of the cleaner (Fig. 2.3), but a clearer difference at high concentrations of cleaner, explaining the significant statistical interaction.

Table 2.1: List of invertebrate and fish taxa collected on control (non-hydrocarbon exposed) oyster shell. A = abundant (> 10 per tray), C = common (1 – 10 per tray), R = rare (< 1 per tray). Oil Sensitivity: Y = yes, significantly affected (out of total number of experiments) or N = not affected

Taxon	Common Name	Abundance	Sensitive to oil?
Arthopoda: Crustacea			
Alpheus heterochaelis	Snapping shrimp	A	Yes (2 of 5)
Callinectes sapidus	Blue crab	C	Yes (2 of 5)
Callinectes similis	Lesser blue crab	C	N
Clibinarius vittatus	Striped hermit crab	R	N
Eurypanopeus despressus	Flat mud crab	C	N
Menippe adina	Stone crab	R	Y (1 of 5)
Panopeus simpsoni	Mud crab	A	N
Palaeomononetes pugio	Grass shrimp	C	N
Penaeus setiferus	White shrimp	R	N
Petrolisthes armatus	Porcelain crab	C	N
Vertebrata: Pisces			
Gobionellus bolesoma	Scaled Goby	R	N
Gobionellus bosc	Naked Goby	C	N
Lagadon rhomboides	Pin fish	R	N
Lutjanus synagris	Lane snapper	R	N
Opsanus beta	Toad fish	R	Y (2 of 5)
Mollusca: Gastropoda			
Littorina irrorata	Marsh periwinkle	C	N
Nassarius acutus	Mud snail	A	Eradicated
Stramonita haemastoma	Oyster drill	R	N

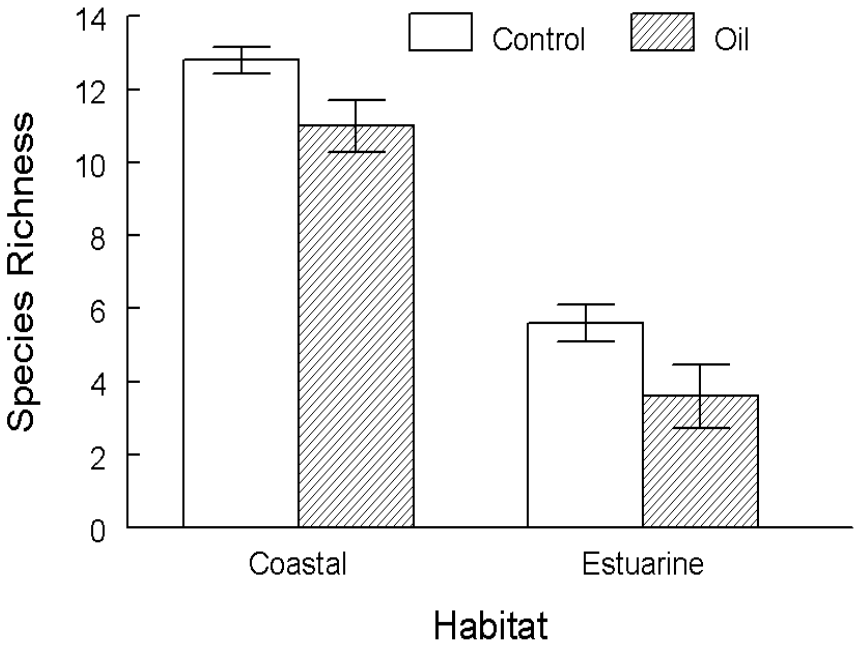

Figure 2.1: Mean number of fish and invertebrate species per tray (± standard error) at two sites along the Louisiana coast, in cultch exposed to two hydrocarbon treatments.

At the highest concentration, the cleaner depressed diversity in trays with control cultch, but increased species richness if cultch had been treated with oil. In contrast, when the total number of organisms per tray was analyzed in the same statistical design, neither of the two main effects (respectively $F_{OIL} = 0.9$, $P = 0.4$, $F_{CLEANER} = 2.3$, $P = 0.2$) nor the interaction ($F_{INT} = 1.0$, $P = 0.4$) were significant.

For the 21 species where individual 2-way analyses (presence of oil x level of cleaner) of variance on abundances were conducted, only two species had reduced abundances on oiled-cultch: the stone crab (*M. adina*) and the toad fish (*O. beta*). The sea squirt (*Molgula manhattensis*) had a significantly higher abundance with joint application of oil and cleaner than when only oil was applied. When the total number of organisms per tray was analyzed, neither of the two main effects nor the interaction was significant.

Effects of heavy crude oil, tidal height and colonization period

Of the three main effects, only tidal height had a significant effect on diversity ($F_{OIL} = 0.7$, $P = 0.7$, $F_{TIDAL} = 14.5$, $P < 0.01$, $F_{COLON} = 0.05$, $P = 0.8$). None of the interactions were significant. On average, diversity was reduced 38 % from 8 species in the sub-tidal to five species at the inter-tidal site (Fig. 2.4). Based on three-way analyses of variance for each taxon, seven species were significantly more abundant in the sub-tidal than in the inter-tidal, while four species showed significant differences in abundance with colonization time.

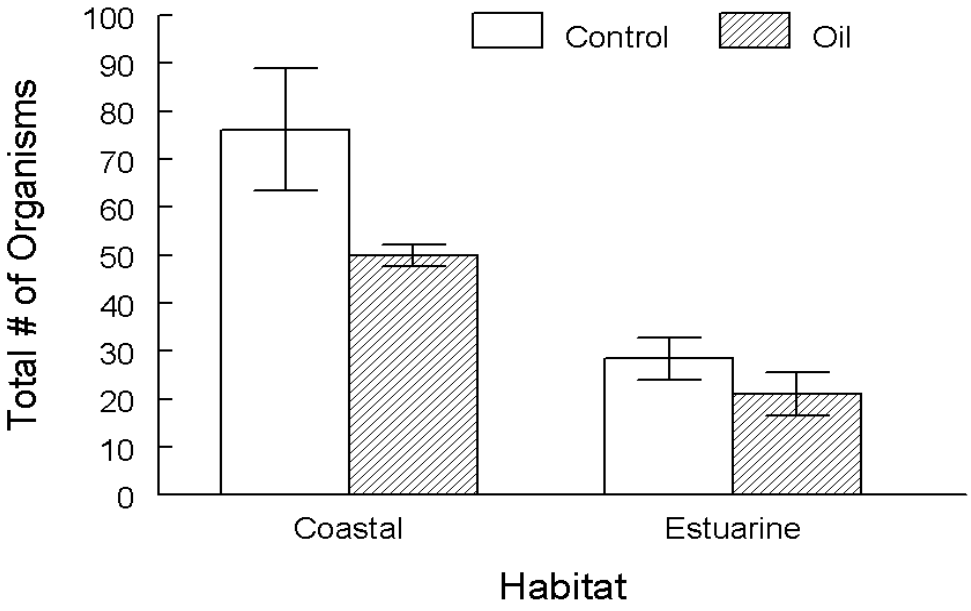

Figure 2.2: Mean total number of organisms per tray (pooled over all taxa) (± standard error) at two sites along the Louisiana coast, in cultch exposed to two hydrocarbon treatments.

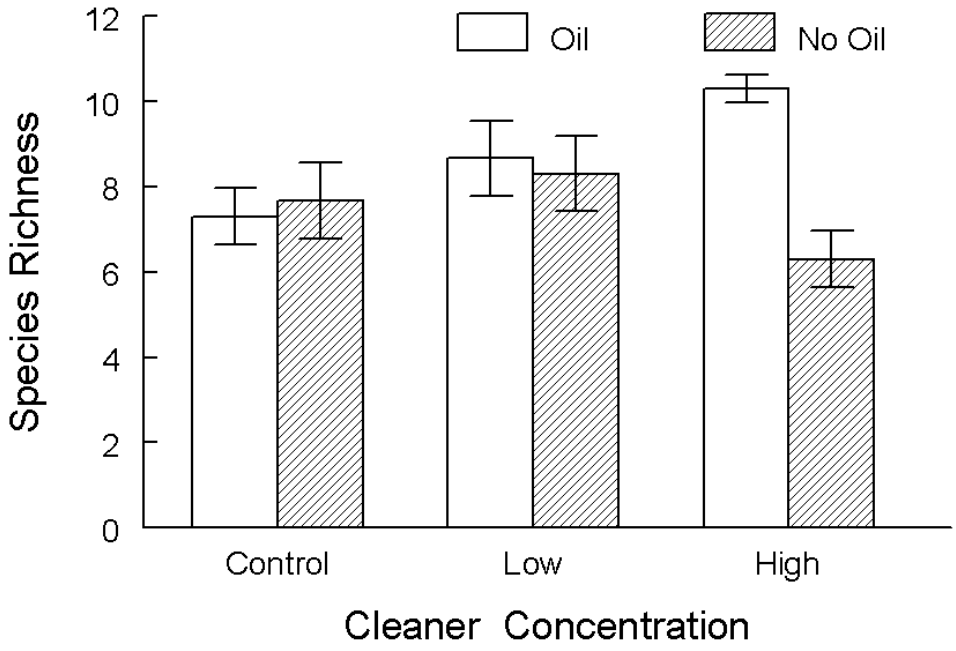

Figure 2.3: Mean number of fish and invertebrate species per tray (± standard error) at the coastal site, exposed to oiled or control cultch and three levels of cleaner.

In contrast, only two species (the snapping shrimp, *Alpheus heterochaelis*, and the lesser crab, *Callinectes similis*) had reduced abundances in the oil-treated cultch. Tidal height ($F_{TH} = 82.5$, $P < 0.01$) was the only variable that significantly affected the total abundance of organisms per tray (with abundance reduced by 59 % at the inter-tidal site, Fig. 2.5). The other two main effects ($F_{OIL} = 1.4$, $P = 0.3$; $F_{CT} = 0.9$, $P = 0.4$) and interactions were not significant.

Seasonal effects

When experiments were compared among seasons, only tidal height had a significant effect on diversity ($F_{TH} = 11.7$, $P < 0.01$) with the sub-tidal site again having more species than the inter-tidal site (Fig. 2.6). However, there was also a significant tidal height by season interaction ($F_{THxS} = 4.8$, $P = 0.04$), because of a higher diversity in summer months, but only at the sub-tidal site. For the individual analyses of variance on each taxon, seven species were more abundant in the sub-tidal than in the inter-tidal, while eight species showed significant differences in abundance with season. Seven species had higher abundances in summer, compared to fall, and one species, the grass shrimp, *Palaemonetes pugio*, had a higher abundance in fall. In contrast, only two species (the snapping shrimp – *A. heterochaelis*, and the lesser blue crab- *C.similis*) had reduced abundances in the oil treated cultch. Tidal height significantly affected the total number of organisms per tray (with abundances again higher at the sub-tidal site), while the other two main effects and interactions were not significant.

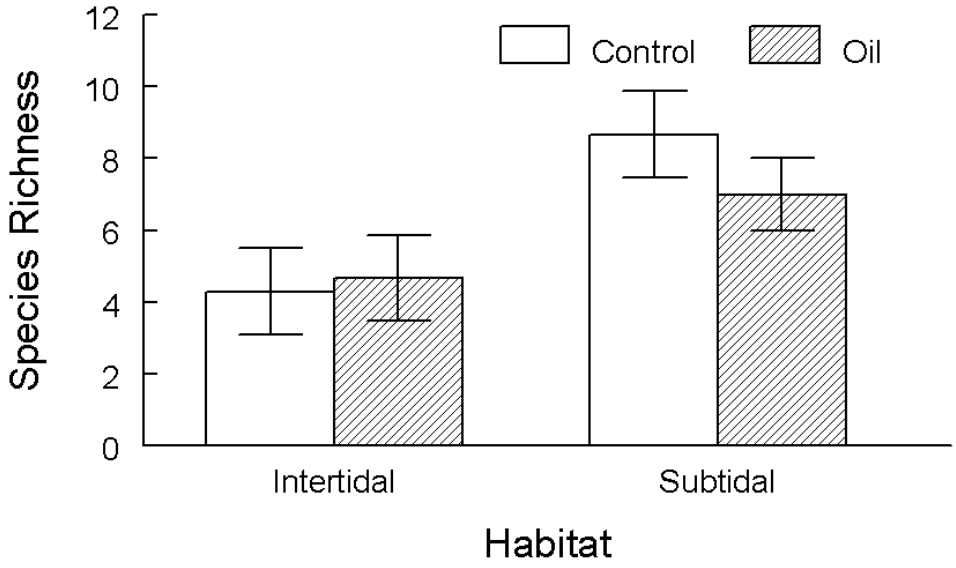

Figure 2.4: Mean number of fish and invertebrate species per tray (± standard error) at two tidal heights at the coastal site, in cultch exposed to two hydrocarbon treatments.

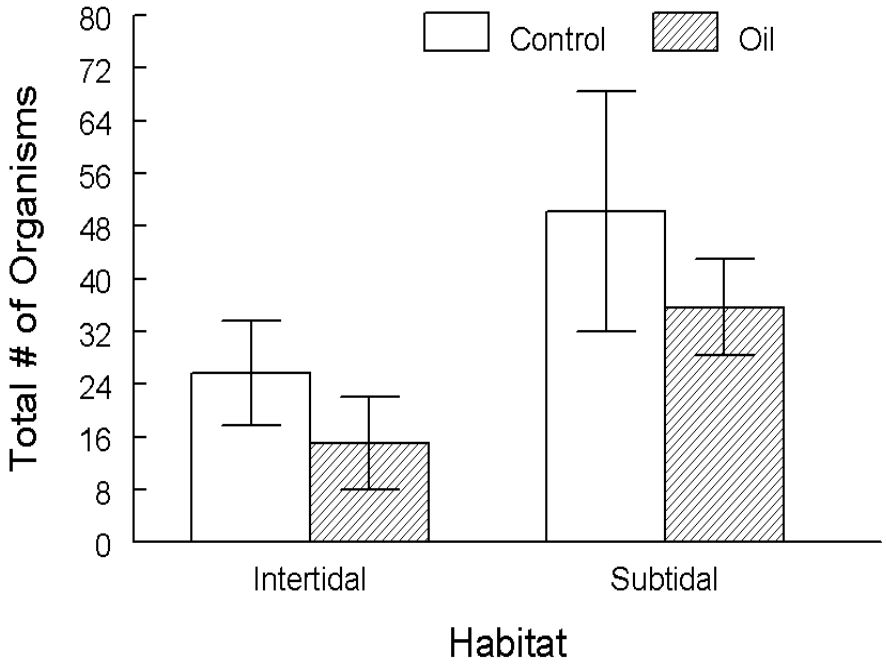

Figure 2.5: Mean total number of organisms per tray (pooled over all taxa) (± standard errors) at two tidal heights at the coastal site, in cultch exposed to two hydrocarbon treatments.

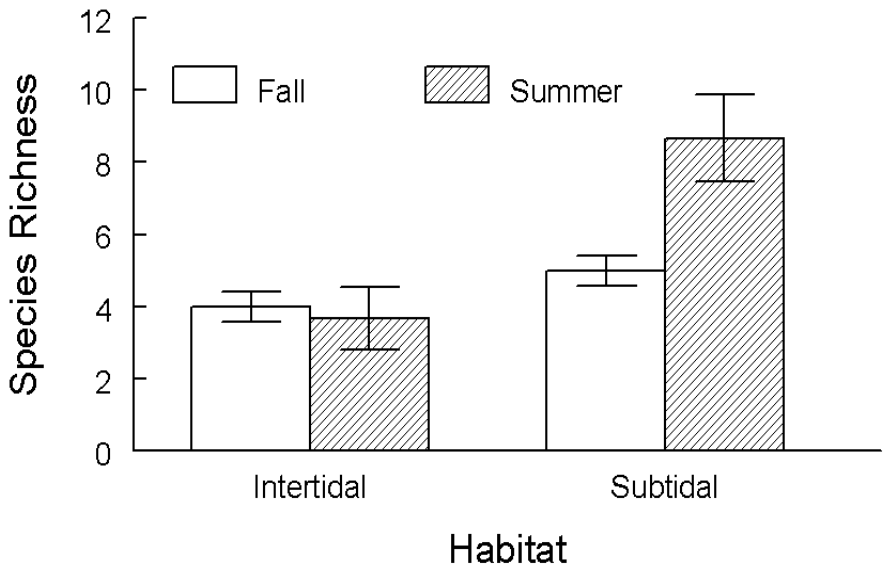

Figure 2.6: Mean number of fish and invertebrate species per tray (± standard errors) at the coastal site, in un-oiled cultch exposed at two tidal heights during two seasons.

Table 2.2: Concentrations of two groups of hydrocarbons determined by GC/MS on oil-soaked shell and control shell, both initially and after one month immersion at three sites, along with values in a reference sample of Venezuelan crude oil

	Total Alkanes (ng/mg)		Total Aromatics (ng /mg)	
	Shell + Mud	Shell	Shell + Mud	Shell
Initial oiled shell	-	1400	-	200
Initial control shell	-	8.4	-	0
Reference oil	-	34000	-	5400
Oiled shell after immersion at coastal inter-tidal site	2.6 (0.2)[1]	80 (5.7)	0.29 (0.1)	8.7 (4.4)
Control shell after immersion at coastal inter-tidal site	0.9	0.05	0.01	0.0
Oiled Shell after immersion at coastal sub-tidal site	27.0 (1.9)	450 (32.1)	18 (9)	120 (60)
Control shell after immersion at coastal sub-tidal site	28.0	18	1.1	0.7
Oiled shell after immersion at estuarine site	81 (6)	50 (3.6)	44 (22)	16 (8)
Control shell after immersion at estuarine site	6.4	13	0.1	0.12

[1] percent of concentration left from concentration on initial oiled shell

Gas chromatography/ mass spectrometry analysis

Considerable loss occurred for both total alkanes and total aromatics on shells immersed at the three sites (Table 2.2). However, the loss was different among the three locations. Averaged over both hydrocarbon groups and shell analyzed with and without mud, about 4.4 % of the hydrocarbons remained (e.g., final concentrations divided by initial concentrations on the shell, Table 2.2) at the coastal inter-tidal site. Average concentrations remaining were much higher, and averaged 25.8 %, at the coastal sub-tidal site. Average remaining concentrations were intermediate, at 9.9 %, at the estuarine site. Hydrocarbon concentrations were also lower, as might be expected, in shell plus mud than in shell with mud removed. Averaged over all three sites and both hydrocarbon groups, remaining concentrations on shell plus mud were 30.9 % of those on shell alone.

Discussion

My results indicate that invertebrate and fish communities on oyster reefs may be more sensitive to variation in salinity and aerial exposure than to hydrocarbon contaminants. Although this result is somewhat surprising, other studies have also indicated that variation in salinity can affect estuarine invertebrate diversity (Wells, 1961). Reduced salinity has also been shown in the laboratory to reduce the scope for growth of blue crabs (Guerin and Stickle, 1997) and Southern oyster drills (Stickle, 1985) as well as affecting the abundance of oysters themselves (Chatry et al.,1983). Oysters again survive best in a salinity range of 5 – 15 PSU, where salinity is high enough for successful recruitment of spat, but low enough to minimize the impact of snail, crab and fish predators (Melancon et al., 1998).

Aerial exposure is also known to limit the foraging abilities of oyster predators (Brown and Stickle, 2002), explaining why many coastal oyster reefs are inter-tidal. Other studies have also indicated higher use of sub-tidal oyster reefs by nekton (Lehnert and Allen, 2002). Undoubtedly, sub-tidal oyster reefs may have higher diversities and abundances of invertebrates and fish as well because constant submergence increases larval settlement rates and survival.

However, as predicted, one of the most-severely hydrocarbon-impacted species was the small detritivorous mollusc, *Nassarius*. Most arthropods and fish (Table 2.1) were either physiologically not affected by hydrocarbons, or possessed mobilities high enough to avoid contaminants and rapidly recolonize trays after hydrocarbons dispersed, also as predicted. Thus even the arthropods and fish that were statistically affected were only depressed in a subset of the experiments.

There are several reasons why hydrocarbon pollutants may not have had a greater impact. First, my artificial reefs were small patches of oil-soaked habitat that could be readily colonized from surrounding areas. A larger-scale oil spill might have a more dramatic impact, especially if it occurred during larval settlement peaks. However, small-scale oil spills (broken pipes, produced water discharges, etc.) are common in the Gulf of Mexico (Rand et al., 1995).

Second, long-term oil production along the Louisiana coastline has probably increased baseline levels of hydrocarbons, possibly "pre-adapting" oyster reefs to hydrocarbon spills. For example, estuarine meiofaunal and fouling communities in Louisiana are less impacted by hydrocarbons than those from Florida marshes with little

oil production infrastructure (Carman et al., 2000; McCoy and Brown 1998). Third, hydrocarbons eventually decay to form bio-films that can actually increase the settlement and growth rates of invertebrates by increasing available food supplies or providing settlement cues (McCoy and Brown 1998; Banks and Brown 2002).

Estuaries also typically display high levels of organic matter in sediment and dissolved particles in water column. Sediment organic material is considered to be the primary factor that controls the bioavailability of hydrophobic contaminants such as PAH (Weston, 1990). So it is possible that after leaching from the shell, the PAH s are adsorbed to organic material in the water column and become less bioavailable.

Finally, the GC/MS analyses also indicate that much of the hydrocarbon coatings initially on shells had dispersed after one month. The lowest concentrations occurred at the inter-tidal site, as would be expected, since shells are exposed to more water flow at inter-tidal sites. Still, over a quarter of the oil remained at the sub-tidal site, indicating considerable coatings of oil remain at least at some sites. When shells are covered with a layer of sediment, as they are on oyster reefs, oil concentrations are also lowered. Thus the loss of oil coatings after a month could also explain the relatively minor effects on diversity and abundance.

My results also suggest that cleaners may lessen any impact of hydrocarbons on oyster reef communities. Cleaners like COREXIT have been reported in earlier studies to have low toxicity (Fingas et al., 1989) and to improve marsh grass and mangrove post-spill survival (Pezeshki et al., 2001).

Based on my results, I predict that the effect of oil spills on estuarine oyster reef communities will depend on the magnitude and location of the spills. Small-scale spills will probably not impact oyster reefs, because of rapid re-colonization rates from surrounding areas and the other factors discussed above. One might expect larger relative impacts at coastal sub-tidal sites, however, because of their higher diversity and less water flow to wash away hydrocarbon coatings. A large-scale oil spill could in contrast seriously impact reefs, particularly if it occurred during peak periods of larval settlement.

Chapter 3
The Effect of Salinity on the Survival and Bioenergetics of Mud Crabs *Panopeus simpsoni* and *Eurypanopeus depressus*

Introduction

Mud crabs of family Xanthidae are common organisms along the Gulf of Mexico, reaching densities as high as $50/m^2$ on oyster reefs (McDonald, 1982). Recent research indicates they have important effects on trophic pathways in reefs. Mud crabs are voracious predators on oyster spat and epi-zoic mussels, and may control oyster recruitment in the absence of fish predators like the oyster toadfish (Grabowski, 2004). Mud crab species often occur in pairs on oyster reefs, and segregate the habitat vertically or by prey type consumed (May, 1974; Meyer, 1994).

Panopeus simpsoni (H. Milne Edwards) and *Eurypanopeus depressus* (Smith) use the oyster reef as a refuge from potential predators and from effects of desiccation at low tide. At high tide, both species utilize a number of reef-associated species as food (McDerott, 1960). Oyster reefs also provide shelter and substrata for as many as 300 other species (Wells, 1961).

Rathbun (1930) subdivided *Panopeus herbstii* into four forms: *typica*, *simpsoni*, *obesa* and *crassa*, with overlapping distributions. Reames and Williams (1984), Sullivan et al. (1984), and Williams (1984) raised these to full species (*P. herbstii*, *P. simpsoni*, *P. obesus* and *P. lacustris*). Williams (1984) considered *P. simpsoni* as endemic to the Gulf of Mexico. *E. depressus* occurs from Massachusetts through Florida to southern Texas, as well as in the West Indies, Uruguay and Bermuda (Williams, 1984). There is often a positive correlation between its abundance and the presence of oyster shell (Ryan, 1956).

Adult *P. herbstii* are predators of the oyster *Crassostrea virginica* (Bahr and Lanier, 1981), the hard clam *Mercenaria mercenaria* (Whetstone and Eversole, 1981), the mussels *Modiolus* spp. and *Brachidontes* sp.(Seed, 1980), and the barnacle *Balanus* sp (Bahr and Lanier, 1981). The smaller *E. depressus* is more omnivorous, with algae and detritus forming the primary diet (Bahr and Lanier, 1981).

Little is known however of what factors determine xanthid distributions. Hydrocarbon contaminants had little effect on mud crab distributions in field colonization experiments (Hulathduwa and Brown, Chapter 2, submitted) in comparison to variation in salinity and aerial exposure. May (1974) also reported that *E. depressus* was more common in more estuarine regions of bays than *P. herbstii* in Alabama. In this study, I therefore systematically studied the role of salinity in the bioenergetics of these two mud crab species.

Estuarine organisms must cope with an array of naturally varying environmental factors, including salinity, temperature and oxygen. While there have been numerous studies on the effect of salinity on tolerance and oxygen consumption in crustaceans (Kinne, 1971) there have been few comparative studies of related crab species from the same general area. While little comparative work has been done with xanthid crabs, the effect of variation in salinity on tolerance and bioenergetics has been studied in blue crabs in the Gulf of Mexico. Blue crabs (*Callinectes sapidus*) are hyper-osmoregulators that adjust easily to salinities ranging from 5 - 30 PSU (Sabourin, 1984). However, scope for growth is more sensitive to low salinity if crabs are collected at higher salinity sites

(Guerin and Stickle, 1992). The lesser blue crab, *C. similis,* is slightly more sensitive to low salinities (as measured by higher respiration rates and lower feeding rates and higher LC_{50}) than the blue crab (Guerin and Stickle, 1997a, b).

Physiological rates can be measured across a salinity gradient and converted to energetic equivalents to determine what effect salinity has on components of the energy budget (Bayne, 1975). Scope for growth, or the energy available for growth and reproduction, can be calculated from these data, and provides an estimate of potential growth over longer periods. Bioenergetic studies are also useful in determining the effects of sub-lethal stressors. Previous studies indicate that decapods adapt to variations in salinity with highly-efficient physiological adjustments (Mangum and Towle, 1977).

To determine the relative sensitivity of *E. depressus* and *P. simpsoni* to salinity, they were exposed to a range of salinities in the laboratory. Hemolymph osmolalities were compared in both species across salinities to see if differences held clues to their distribution. I also determined the salinity tolerance of the two mud crab species and investigated the effect of salinity on their bioenergetics, using scope for growth as an indicator of sub-lethal stress. The results are compared to similar studies on other crab species and discussed as they relate to the field distribution.

Materials and Methods

Collection and maintenance of crabs

The two mud crab species (~ 12 to 26 mm carapace width) were collected from an inter-tidal oyster reef near the LUMCON (Louisiana Universities Marine Consortium)

laboratory at Port Fourchon, Louisiana, USA, in June 2004. This site is in the same salt marsh (29° 2′N; 90° 1′W) described in Guerin and Stickle (1992). Salinities fluctuate anywhere from 10 – 30 PSU at this site, about 10 km inland from the Gulf of Mexico. I filled mesh bags (0.67 x 0.33 m, mesh size = 1.6 cm) with oyster shell (15 – 25 cm shell length) and set them out in the oyster reef for a month. Bags were then carefully retrieved and placed in large plastic tubs to avoid loss of smaller crabs. The shell was washed over a series of sieves (1 – 2 mm) and the crabs were hand-collected. Salinity was 26 PSU at the time of collection, and water temperature was 29° C.

All crabs were transported to Louisiana State University, Baton Rouge. The two species were held separately in 38- liter aquaria equipped with under-gravel filters. Water was set at ambient salinity (26 PSU) using artificial sea salts (Instant Ocean, Aquarium Systems Inc.). Crabs were isolated from each other in individual chambers (10 X 8 X 6 cm^3) in larger (50 X 40 X 6 cm^3) plastic boxes, to prevent cannibalism and allow measurement of feeding rates. Water temperature was maintained at 24°C and the tanks were kept in constant light. Treatment salinities were reached by step-wise acclimation (2 to 4 PSU d^{-1}) from the initial salinity.

Basic technique

Salinity tolerance was examined by exposing crabs to seven salinities, and crabs were monitored for mortality over a 28 d period. The LC_{50} was used as a measure of tolerance to low salinity. Measurements of oxygen consumption, ammonium excretion and food consumption were also used to examine the sub-lethal effects of salinity. These energy-budget components were used to determine scope for growth for each species at

each salinity. Hemolymph osmolality also was assessed to examine if the osmoregulatory ability of the two species differs.

Energy budget

Scope for growth is the energy accumulated for somatic growth and reproduction. It is determined from the balanced energy equation of Winberg (1960):

$$C - F = Ab = R + U + P, \quad (1)$$

Where C = energy consumed as food, F = energy lost as feces, Ab = energy absorbed from food consumed, R = energy lost as respiration, U = energy lost as excretion, and P = energy accumulated for growth and reproduction. Scope for growth (P) was estimated as $P = Ab - (R + U)$.

Experimental design

The bioassay consisted of 10 crabs of each species at each of 7 salinities (1, 2.5, 5, 10, 17.5, 22.5 and 25 PSU). Crabs were monitored for mortality over a 28 d period, beginning on the day that the final target salinity was reached. Survival data was used to calculate LC_{50}, the salinity resulting in 50% mortality. Daily LC_{50} values were calculated using procedure Probit of SAS (1985).

Measurements of oxygen consumption, ammonium excretion and food consumption were made for 8 crabs of each species at 4 treatment salinities (7.5, 12.5, 17.5 and 25 PSU) over a 14 d period. These crabs were independent from those used for determination of survival rates. Measurements began (Day 0) 1 wk after target salinities had been reached, and were carried out on Days 0, 7 and 14. Day 14 of the bioassay

portion of the study is equivalent to day 7 of the bioenergetics portion of the study, due to The 1 wk acclimation period employed in the latter, but not in the former.

Consumption rates of crabs exposed to the four treatment salinities (7.5, 12.5, 17.5 and 25‰) were determined by measuring feeding rate each day of the week prior to Days 0, 7 and 14, to obtain an average daily feeding rate for each time. Crabs were fed a commercially available fish food (Tetra Exotic^{TM}, sinking mini sticks) daily. The uneaten portion of the food was removed daily and weighed, and a new portion was introduced into the chamber with the crab. Food consumption was converted to energetic equivalents (*C* in Eq.1) using conversion factors of 5.205 cal mg^{-1} dry weight (determined by bomb calorimetry) and converted to joules.

Absorption efficiency was determined by the ash-ratio method of Conover (1966). Feces from crabs at each salinity were collected, dried and ashed along with food samples. Absorption efficiency was calculated using the formula:

$$(F - E) * [(1 - E) F]^{-1} * 100 = Ab \qquad (2)$$

Where F = ratio of ash-free dry weight to dry weight in the food, and E = ratio of ash-free dry weight to dry weight in the feces. Energy absorbed from food (Ab) was calculated by multiplying energy consumed as food (C in Eq. 1) by the absorption efficiency.

Oxygen consumption rates of these same crabs (and same treatment salinities, N = 8 crabs of each species) were measured on Day 14 using flow-through respirometry (Stickle et al. 1985). Crabs were enclosed in 250 ml glass respiration-chambers through

which water of the appropriate salinity flowed at ≈ 10 ml per minute (8 chambers contained crabs and two were empty control chambers). Crabs were left in the chambers for an acclimation period of 2 h, and out-flow water samples were collected anaerobically with a syringe and injected directly into an oxygen electrode to read oxygen partial pressure. Another reading was repeated after the initial reading. Oxygen consumption rates were converted into energetic expenditure (*R*) using the oxycalorific value of 4.8 cal ml O_2 consumed $^{-1}$ (Crisp, 1971) and multiplied by 4.19002 J cal^{-1}.

Ammonium-excretion rates were measured on Day 14 in conjunction with respirometry in outflow water from respiration chambers. Ammonium levels were determined using Grasshoff and Johannsen's (1972) modification of the phenol-hypochlorite method of Solorzano (1969). Ammonium excretion rates were converted to energy expenditure rates (*U*) using the factor of 0.0832 cal μ*M* $ammonium^{-1}$ (Elliott and Davison 1975). Crabs were weighed following each molt and weekly so that the various physiological rates could be standardized (J standard 1 g $crab^{-1}$ d^{-1}).

Hemolymph osmolality

To assess hemolymph osmolality, seven crabs of each species were taken from each salinity level. Two replicate10 μl hemolymph samples were withdrawn through the arthrodial membrane above the 5th walking leg of each crab by using ultra-micro pipettes. Samples were immediately centrifuged in polyvinyl tubes for five minutes at 10,000 x g. The osmolality of hemolymph supernatant and water samples were determined with a Wescor vapor-pressure osmometer (Model 5100B).

Statistical analyses

To remove the effects of body weight on physiological rates, rates were standardized to a 1 g ash-free dry body weight crab. Regressions of all physiological rates with ash-free dry body weight were compared among salinities using analysis of covariance. If regressions were significant, and the slopes differed from zero, then weight affected the rate. If the slopes differed among salinities then this size dependency effect differed among salinity treatments. Different regressions were used for each salinity if the latter case were true. Otherwise, data were combined to obtain a common regression. Adjusted physiological rates were calculated using the formula:

$$Y' = Y - [b\,(WT - \bar{W}\ \bar{T})]$$

from Neter and Wasserman (1974), where, Y′ = adjusted (weight –specific) rate, Y = unadjusted rate, b = slope of the regression of unadjusted rate and ash-fee dry weight, WT = ash-free dry weight of the individual crab, and $\bar{W}T$ = mean ash-free dry weight of all crabs. Y′ was then divided by WT to obtain an adjusted rate per gram ash-free dry weight. If the regression effect were insignificant, then the unadjusted rate was simply divided by ash-free dry weight for each crab.

Two-way ANOVA (SAS Institute Inc.1985) was used to test for the effects of salinity and species once the adjusted (weight-specific) rates were obtained. Individual two-way ANOVAs were performed on oxygen consumption, ammonium excretion, amount of ingested food, energy absorbed, energy expended ($R + U$) and scope for growth. Tukey's Studentized Range (HSD) test was used to test for differences among individual treatment means.

Results

Survival

The 28 d LC_{50} of *E. depressus* was 0.19 PSU compared with 6.97 PSU for *P. simpsoni*. All individuals of both species survived the 25, 22.5 and 17.5 PSU salinity treatments for 28 d. *P. simpsoni* exhibited 7, 14, 21 and 28 d LC_{50} s of 5.95, 6.29, 6.97 and 6.97 PSU respectively (Fig 3.1). *E. depressus* exhibited 7, 14, 21 and 28 d LC_{50} s of 0.12, 0.19, 0.19 and 0.19 PSU respectively (Fig 3.1). These differences in LC_{50} values indicate *E. depressus* was obviously more tolerant of low salinities than *P. simpsoni*.

Energy consumption and absorption

The amount of ingested food was significantly reduced at low salinity in both species, but there was no difference in response between species (Table 3.1). The amount of food ingested at 7.5 PSU was approximately 25 % less than at 17.5 PSU in both species. The amount of energy absorbed also decreased with decreasing salinities. Energy absorbed did not significantly differ at 25 and 17.5 PSU for either species. At lower salinities (12.5 and 7.5 PSU) absorbed energy decreased significantly. Absorption efficiencies of *P. simpsoni* at the four salinities were as follows: 7.5 PSU = 49.8%, 12.5 PSU = 62.1%, 17.5 PSU = 71.4%, 25 PSU = 67.8%. Absorption efficiencies of *E. depressus* at the four salinities were as follows: 7.5 PSU= 52.3%, 12.5 PSU = 63.6%, 17.5 PSU = 71.6%, 25 PSU = 66.4%. For both species, the highest absorption efficiency was seen at 17.5 PSU and the lowest was at 7.5 PSU.

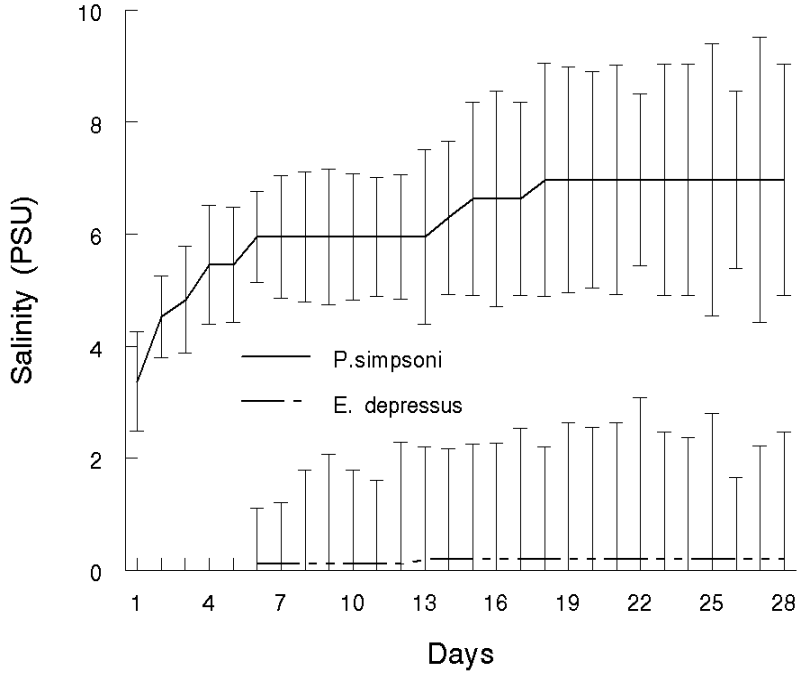

Figure 3.1: Tolerance of *P.simpsoni* and *E. depressus* to salinity, expressed as LC_{50} s through 28 days.

Energy absorption rates (*Ab* = *C* X absorption efficiency) also declined at salinities below 17.5 PSU and there was again no significant difference between the responses of the two species. Absorption rates were highest at 17.5 PSU and there was approximately a 50 % drop at the lowest salinity (7.5 PSU) for both species. Ingestion averaged 3.74 and 3.70 times energy expenditure (*R* + *U*) for *P. simpsoni* and *E. depressus*, respectively. Energy absorption rates averaged 2.42 and 2.41 times energy expenditure (*R* + *U*) for *P. simpsoni* and *E. depressus* respectively.

Energy expenditure

Total energy expenditure rate (*R* + *U*) was highest at the lowest salinity (7.5 PSU) as were energy expenditure due to both respiration (*R*) and ammonium excretion (*U*) for both species (Table 3.1). Total energy expenditure was approximately in 35 % higher at the lowest salinity (7.5 PSU) compared with 25 PSU. Most of the energy expenditure (89.4% average for *P. simpsoni* and 89.6% for *E. depressus*) was due to respiration, with the remainder due to excretion. The effect of salinity on total energy expenditure was significant for both species. However, there was no significant difference in the response in total energy expenditure to salinity between the two species. The effect of salinity on *U* was not significant, but the effect of salinity on *R* was significant. For both species, the amount of energy expenditure relative to the amount of both energy consumption and absorption increased as salinity decreased and was greatest at 7.5 PSU.

Table 3.1 Effect of salinity on energy-budget components of *P. simpsoni* and *E. depressus* expressed as kJ g^{-1} ash-free dry wt d^{-1}. [** highly significant effect of salinity (*S*) or Species (*Sp*) revealed by ANOVA s ($p < 0.01$); * significant ($p < 0.05$); *NS* not significant ($p > 0.05$); superscripts represent Tukey's Studentized Range (HSD) test for comparision of means between salinity and species; means bearing same letter are not significantly different]

Energy allocation		Salinity			Effect		Interaction
	7.5	12.5	17.5	25	Sal	Sp	
Ingested (C)							
Ps	1.33^{a}	1.51^{b}	1.85^{c}	1.82^{c}	*	NS	NS
Ed	1.36^{a}	1.52^{b}	1.80^{c}	1.78^{c}	*	NS	NS
Absorbed (Ab)							
Ps	0.66^{a}	0.94^{b}	1.32^{c}	1.24^{c}	*	NS	NS
Ed	0.71^{a}	0.97^{b}	1.29^{c}	1.18^{c}	*	NS	NS
Respired (R)							
Ps	0.502	0.374	0.365	0.366	*	NS	NS
Ed	0.500	0.376	0.367	0.376	*	NS	NS
Excreted (U)							
Ps	0.057	0.049	0.042	0.043	NS	NS	NS
Ed	0.057	0.048	0.041	0.041	NS	NS	NS
% due to respiration							
Ps	89.8	88.4	89.7	89.5			
Ed	89.9	88.5	90.0	90.0			
% due to excretion							
Ps	10.2	11.6	10.3	10.5			
Ed	10.1	11.5	10.0	10.0			

Scope for growth

Scope for growth (*P*) declined below 17.5 PSU for both species (Fig 3.2 and 3.3). There was no significant difference in *P* among the two salinity treatments of 17.5 PSU and 25 PSU for either species. Scope for growth was lowest at the lowest salinity (7.5 PSU) for both species, with approximately 80% drop from 17.5 PSU. Even at 7.5 PSU scope for growth remained positive. There was no significant difference in *P* between the two species.

Hemolymph osmolality

Both *P. simpsoni* and *E. depressus* are hyper-osmoregulators maintaining a higher osmotic concentration in their bodies than the ambient sea water. The hemolymph osmotic concentration still however decreased with decreasing salinity (Fig. 3.4). Their osmoregulatory ability did not differ significantly between species (P = 0.3879).

Discussion

I found that *E. depressus* was more tolerant of low salinity than *P. simpsoni*. However, both species could survive a salinity of 6 PSU for at least a week. Wells (1961) has determined that the mud crab *P. herbstii* could survive a salinity of 4 PSU for at least 5 days, and Davies (1974) found that the range of 2 to 40 PSU could be tolerated for at least 15 days.

May (1974) reported that *E. depressus* was less affected by low salinity than *P. herbstii* in Alabama estuaries. Both species were more abundant in high salinity areas, but *P. herbstii* was rare where salinity averaged below 15 PSU.

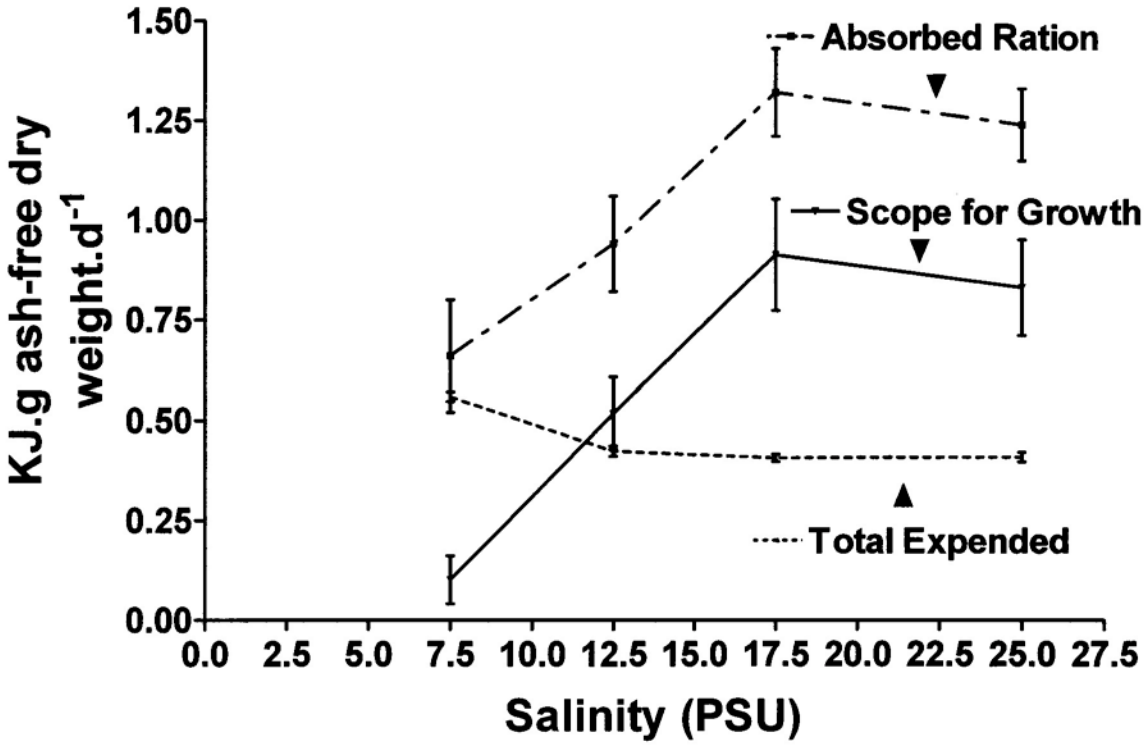

Figure 3.2: Absorbed ration, total energy expenditure and the scope for growth of *P. simpsoni* exposed to different salinity levels.

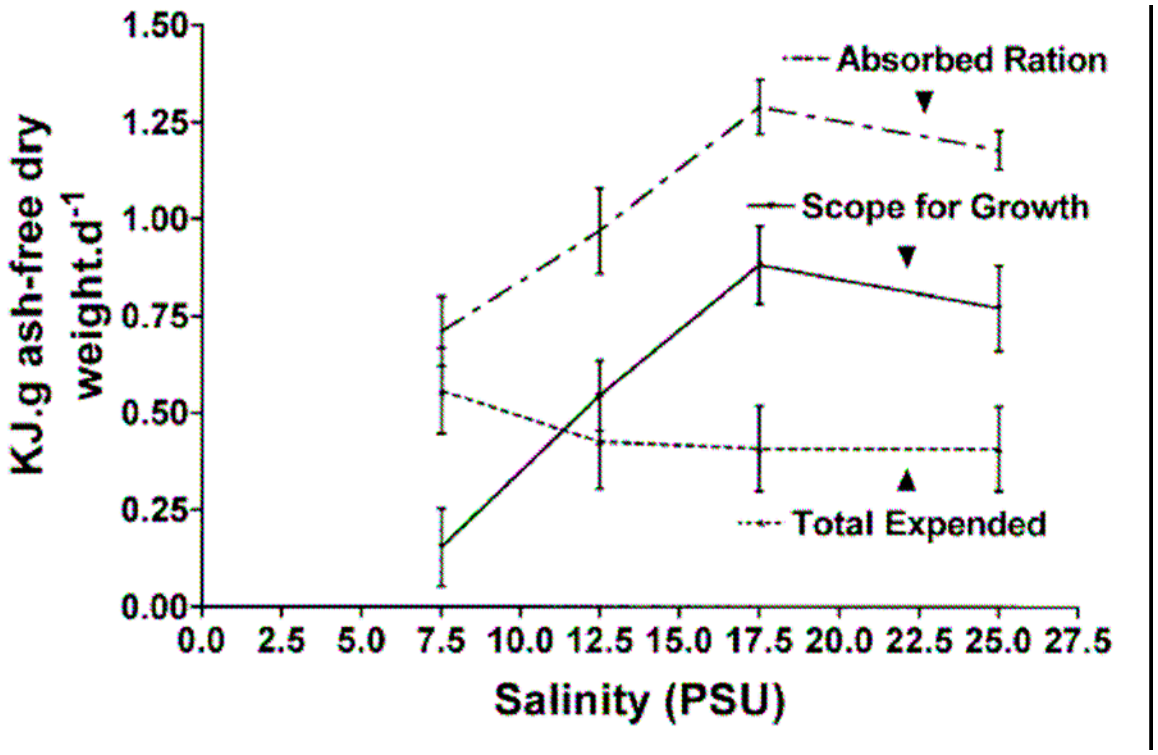

Figure 3.3: Absorbed ration, total energy expenditure and the scope for growth of *E. depressus* exposed to different salinity levels.

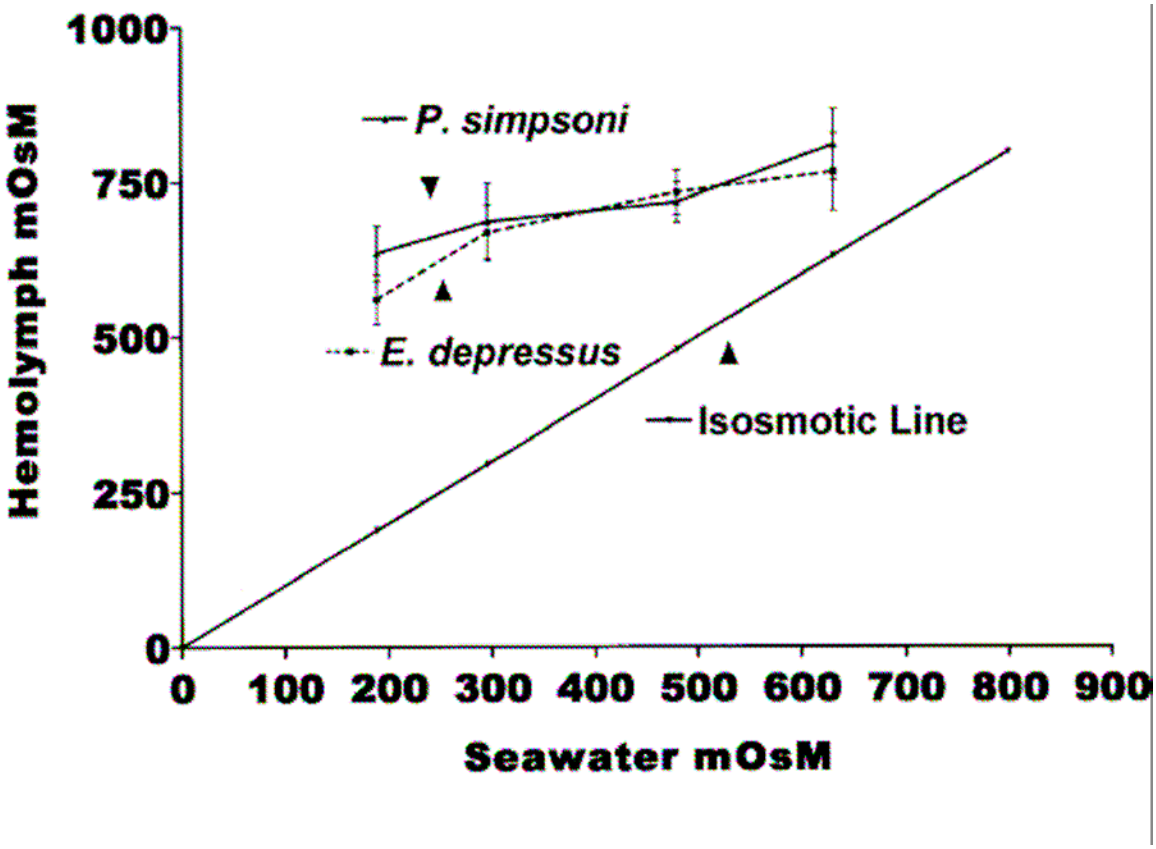

Figure 3.4: Hemolymph osmolality of *P. simpsoni* and *E. depressus* exposed to different salinities.

The ability of *E. depressus* to tolerate lower salinities than *P. simpsoni* indicated here may explain this distribution pattern. However, there may be other important factors in explaining the distribution pattern of these crabs. Shumway (1983) also reported *P. herbistii* as common only in high salinity areas despite its tolerance of low salinity. *E. depressus* exceeds most other euryhaline crustaceans with regard to salinity tolerance (Davenport, 1972; Knowlton and Kirby, 1984; Knowlton and Schoen, 1984), although the blue crab *C. sapidus* tolerates even lower salinities (surviving at 0 PSU) (Guerin and Stickle, 1992).

For both crab species, total energy expenditure ($R + U$) increased with decreasing salinity. Respiration was the largest component of energy expenditure in *P. simpsoni* and *E. depressus*. This is also the case with *C. sapidus* (Guerin and Stickle, 1992) and *C. similis* (Guerin and Stickle, 1997). Respiration rate and ammonium excretion rate both increased as salinity decreased in both mud crab species. The increase in respiration rate with decreasing salinity occurs in most marine invertebrates (Findley et al., 1978; Guerin and Stickle, 1997), although in some cases no relationship is apparent (Sabourin, 1984; Guerin and Stickle, 1992). Mangum et al (1976) found that the excretion rates of blue crabs increased with decreasing salinity. Shumway (1983) reported increased respiratory rates in four Brazilian crab species including *P. herbistii* when exposed to dilute sea water. *P. herbstii* in that study showed no change in oxygen consumption in dilutions as low as 60% sea water (20.4 PSU) but then oxygen consumption increased dramatically from 40-0% sea water (13.6 PSU), reaching a maximum in 0% sea water.

The two crab species in my study demonstrated a similar trend with significant increases in energy expenditure occurring only at salinities lower than 12.5 PSU. Dimock and Groves (1975) also reported that the oxygen consumption rate of the mud crab *P. herbistii* increased as salinity decreased. Since exposure to reduced salinity results in an increased osmotic gradient across the body wall of an osmoregulating organism, the energy requirements of regulation could contribute to enhanced oxygen consumption. Flemister and Flemister (1951) and Schlieper (1971) also found increased metabolic rate correlated with increased osmotic work.

Salinity also affected energy absorption (calculated from food consumption and absorption efficiency) for both crab species, as in most marine invertebrates exposed to gradients of environmental factors (Shirley and Stickle 1982, Stickle et al., 1985; Stickle and Bayne, 1987). As a result, scope of growth paralleled energy absorption. Energy absorption was lowest at 7.5 PSU, resulting in the lowest scope for growth at this salinity for both species. High energy expenditure at 7.5 PSU (relative to that at higher salinities) also contributed significantly to low scope for growth at 7.5 PSU. Highest energy absorption rate and scope for growth occurred at 17.5 PSU. There was no significant difference in scope for growth above 17.5 PSU for either species, indicating that both species operate at their physiological optimum at this salinity range, accumulating maximum energy for growth.

Furthermore, having a low but still positive value for scope for growth at 7.5 PSU indicates that both species can survive and grow even at this low salinity. Both *P. simpsoni* and *E. depressus* may be exhibiting a trade-off, utilizing the increased resources

of low-salinity inshore waters at the expense of sub-optimal physiological function. Extended exposure to low salinities, however, could result in increased stress and lower growth, explaining why these crabs may not remain in low salinity waters for long.

Semiterrestrial, inter-tidal and brackish water crustaceans often demonstrate the ability to discriminate between salinities or to move along salinity gradients (Davenport and Wong, 1987), and such abilities allow animals to move from areas of sub- optimal salinity to more favorable conditions. Both crab species were hyper osmoregulators, but their osmorgulatory ability did not differ. McDonald (1982), documented that a combination of trophic and microhabitat factors could explain the co-occurrence of *P. herbstii* and *E. depressus* in South Carolina. In this study, *E. depressus* was capable of surviving lower salinities than *P. simpsoni.* However, the physiological responses studied here do not significantly differ between the two species and cannot therefore explain the different tolerances. Other physiological and ecological factors such as competition may have to be studied to more fully understand the underlying mechanisms limiting the distribution of these crab species.

Chapter 4
Competitive Ability and Predation Risk in the Mud Crabs *Panopeus simpsoni* and *Eurypanopeus depressus*

Introduction

Marine crustaceans vigorously defend shelter and food resources (Steger, 1987) and the availability of refuges influences the density and size structure of many marine crustacean populations (Caddy and Stamatopoulas, 1990; Beck, 1997). Refuges can positively affect populations by reducing predation (Holt, 1984), ameliorating physical disturbances (Howard and Nunny, 1983) or by reducing physiological stress (Bertness, 1981). Shelter availability regulates abundance, density and size structure of local populations in hermit crabs (*Clibanarius albidigitus*, *Calcinus obscurus*, *Pagurus* sp.; Bertness, 1981), stone crabs (*Menippe adina,* Beck, 1995; Shervette et al. 2004), American lobsters (*Homarus americanus*; Wahle and Steneck, 1991), and spiny lobsters (*Panulirus argus*, Eggleston and Lipcius, 1992). Heck and Coen (1995) linked refuge availability to survival of juvenile blue crabs, *Callinectus sapidus,* in the Gulf of Mexico. Refuge limitation acting on a specific size class often creates a population bottleneck that limits the overall production of a crab population.

The xanthid mud crabs *Panopeus simpsoni* (H. Milne Edwards) and *Eurypanopeus depressus* (Smith) are ubiquitous inhabitants of oyster *(Crassostrea virginica*) reefs along the SE Atlantic and Gulf Coast lines (McDonald, 1982). Little is known of factors determining xanthid crab distributions. McDonald (1982) concluded a combination of trophic and microhabitat factors explained the co-occurrence of *P. herbstii* and *E. depressus* on oyster reefs. In my earlier research, I found that hydrocarbon

contaminants had little effect on mud crab distributions in field colonization experiments, in comparison to variation in salinity and aerial exposure (Hulathduwa and Brown, Chapter 2, submitted). *E. depressus* is more common in upper, more estuarine regions of bays than *P. herbstii* in Alabama (May, 1974). *E. depressus* is also capable of surviving lower salinities than *P. simpsoni* (Hulathduwa et al., Chapter 3, submitted) although scope for growth, calculated from energy absorption and energy expenditure, does not differ between the species. Brown et al. (2005) found that adult *E. depressus* were behaviorally dominant over adult *P. simpsoni* for both food and shelter resources, and also demonstrated that the dominance hierarchy predicted resource-holding potential. Both *E. depressus* and *P. simpsoni* spend their whole post-larval life cycle on oyster reefs, and *P. simpsoni* can reach 5 cm in carapace width, while adult *E. depressus* only reach 2 cm carapace width. These two species often co-occur and differences in vertical distribution, feeding niches or salinity tolerances may limit competition (May, 1974; Meyer, 1994).

Oyster reefs, sea grass beds and salt marshes have high infaunal densities because habitat complexity provides refuge from predation (Summerson and Peterson, 1984). Oyster reefs provide refugia for a diverse assemblage of fishes, crustaceans, polychaetes and mollusks (Coen et al., 1999). Xanthid crabs are important predators of juvenile oysters (Meyer, 1994), and are important prey for the oyster toadfish, *Opsanus tau* (Wilson et al., 1982). Grabowski (2004) found that oyster toadfish reduced xanthid crab abundances and indirectly facilitated oyster spat survival, although vertical habitat complexity on oyster reefs weakened the trophic cascade.

Selective feeding by crabs is an important factor structuring marine communities (Hines et al. 1990). The common blue crab *Callinectes sapidus* is widespread along the Southeastern coastline of the USA (Williams, 1984) and is an important predator of several shell fish (Seed, 1993).

Since *E. depressus* and *P. simpsoni* often show non-overlapping distributions in estuaries, with the behaviorally dominant *E. depressus* more common in more estuarine areas, I decided to further explore relationships between salinity tolerance, resource holding potential (RHP), and predator risk in determining the distributions of these two crabs. In laboratory experiments, I first studied how the use of shelters in short supply was related to both salinity and dominance. Individuals of both species were held with a shortage of spatial refugia to see which species would successfully defend shelters at both an average and a low salinity. I predicted that *E. depressus* would have greater RHP at both salinities because of its greater behavioral dominance.

In a second set of laboratory experiments, I looked at the relationship of shelter use, salinity and predation risk. Both xanthid crabs were again held with a limited number of refugia, at both high and low salinities, and survival was documented in the presence of a blue crab predator. I hypothesized that *E. depressus*, because of greater RHP, would have higher survivorship rates.

Materials and Methods

Collection and maintenance of crabs

The two mud crab species (varying from ~ 12 to 35 mm in carapace width) were collected from an inter-tidal oyster reef near the LUMCON (Louisiana Universities

Marine Consortium) laboratory at Port Fourchon, Louisiana, USA (29° 2′N; 90° 1′W) in July, 2005. I filled mesh bags (0.67 x 0.33 m, mesh size = 1.6 cm) with oyster shell (15 – 25 cm shell length) and set them out on the oyster reef for a month to collect crabs (Stuck and Perry, 1992). Bags were then carefully retrieved and placed in large plastic tubs to avoid loss of smaller crabs. The shell was washed over a series of sieves (1 – 2 mm) and the crabs were hand-collected. Salinity was 26 PSU at the time of collection, and water temperature was 29° C.

All crabs were transported to Louisiana State University, Baton Rouge. In the laboratory, crabs were identified to genus and species using taxonomic keys (Felder, 1973; Hopkins et al., 1989) and carapace widths (including anterior-lateral spines) measured to the nearest 0.1 mm with dial calipers. Prior to experiments, the two species were held separately in 38-liter aquaria equipped with under-gravel filters. *P. simpsoni* was marked with a dot of white paint on the carapace for easy identification. Crabs were held at ambient salinity (26 PSU) using artificial sea salts (Instant Ocean, Aquarium Systems Inc.). Crabs were held in isolation from each other in individual chambers (10 X 8 X 6 cm^3) in larger (50 X 40 X 6 cm^3) plastic boxes in 40 L aquaria, to prevent cannibalism. Water temperature was maintained at 24°C. Blue crabs (*C. sapidus*) were trapped in baited-crab traps at the Port Fourchon laboratory in fall 2005. Individual blue crabs were held in 38-liter aquaria equipped with under-gravel filters.

Shelter use experiments

To determine if the two species differed in resource holding potential, and if resource holding was dependent on salinity, I performed a laboratory experiment in the

fall of 2005. Eight 19-L aquaria with under-gravel filters were used in this experiment; four had a salinity of 26 PSU and four were maintained at 7.5 PSU. 25 PSU was selected as high salinity while 7.5 PSU is near the lower salinity tolerance limit of *P. simpsoni*. (Hulathduwa et al., submitted). Five PVC pipes (5 cm in length and 2.5 cm in diameter), with one side covered with 1 mm Vexar® mesh hot-glued to the end of the cylinder, were placed in each aquarium as refugia. Small portions of a commercially available fish food (Tetra Exotic™, sinking mini sticks) were placed in shelters initially, and if empty when the shelters were later examined. Five crabs of each species were then placed in each aquarium and refugia were checked at 9am, 2 pm and 7 pm daily for three days, and the species occupying the shelters noted.

Shelter use and predation risk

To determine if resource holding potential for refugia influenced predation risk, I performed a second laboratory experiment immediately following the first in late fall 2005. Individuals of both mud crab species were held with a blue crab (*C. sapidus*) again with a limited number of refugia. Ten 40-L aquaria equipped with under-gravel filters were used in these experiments. To determine the role of ambient salinity, five aquaria were again held at 26 PSU and the other five at 7.5 PSU. There were five shelters and five crabs of each species per tank. A single male blue crab (carapace width 12-14 cm) was introduced 3 hours after the mud crabs were introduced, to allow individual mud crabs to initially find and occupy shelters. The numbers of crabs of each species surviving in each tank were then recorded after 4 hr, 10 hr, 24 hr and 48 hr from the time of blue crab introduction.

Statistical analyses

To determine if there was a difference between species in the average number of crabs occupying the shelters in the first experiment, a 2-way analysis of variance was performed with species and salinity the main effects. Tukey's *a posteriori* test was used to identify which means differed (PC SAS Version 9; SAS Institute Inc., 1988).

To determine whether the number of crabs surviving blue crab predation differed between species and salinities over time, a repeated measures analysis of variance was performed (the arrangement of treatments was two species x two salinities, with the 4 observations of each tank as the repeated measure). Percentages were arcsin-square root transformed to solve normality and variance homogeneity problems (Sokal and Rohlf 1981).

Results

Shelter use experiments

Shelter occupancy significantly differed between species ($F = 143.63$; $P < 0.0001$) and the two salinity levels ($F = 135.84$; $P < 0.0001$). *E. depressus* was clearly dominant over *P. simpsoni* in occupying shelters at both salinity levels (Fig. 4.1). Shelter occupancy was respectively 38% and 63% lower by *P. simpsoni,* compared with *E. depressus*, at 25 and 7.5 PSU. For both species, fewer crabs occupied shelters at the lower salinity. *E. depressus* and *P. simpsoni* exhibited 38% and 60% lower occupancy respectively at 7.5 PSU.

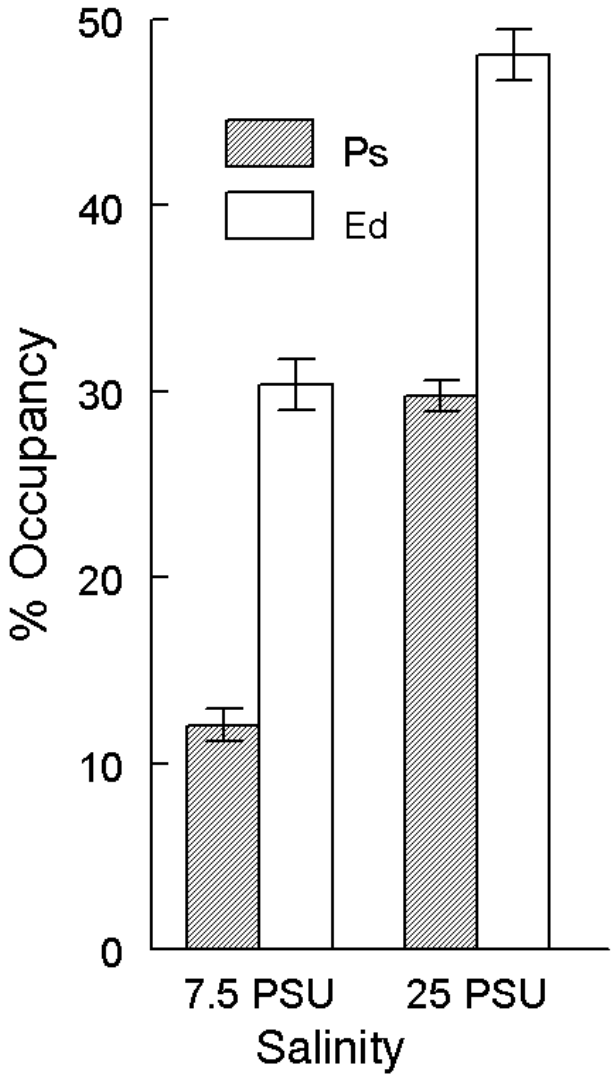

Figure 4.1: Percentage shelter occupancy (± SE) by *P. simpsoni* and *E. depressus* at two salinities.

Predation risk experiments

Survival of both crab species declined significantly with time due to predation by blue crabs (Table 4.1). Less than 20% and approximately 25% of the initial population survived after 48 hours for *P. simpsoni* and *E. depressus,* respectively (Fig. 4.2). Survival also differed significantly between the two species (Table 4.1) with *E. depressus* exhibiting higher survival. Salinity had a marginally significant effect, but there was no significant interaction between species and salinity, indicating that both species survived similarly at both salinity levels. There was also no significant interaction between time and species indicating that both species had similar time-dependent survival trends.

Table 4.1: Statistics for repeated measures ANOVA for percentage survival of *P. simpsoni* and *E. depressus* exposed to blue crab predation, measured over 5 time intervals at two salinity levels.

Contrast	Wilk's Lambda	F	P
MANOVA tests			
Time	0. 012	268.4	<0.0001
Time X Species	0.730	1.2	0.36
Time X Salinity	0.774	0.95	0.47
Time X Species X Salinity	0.906	0.34	0.85
Between subjects tests			
Species	___	5.75	0.03
Salinity	___	3.24	0.09
Species X Salinity	___	0.09	0.77

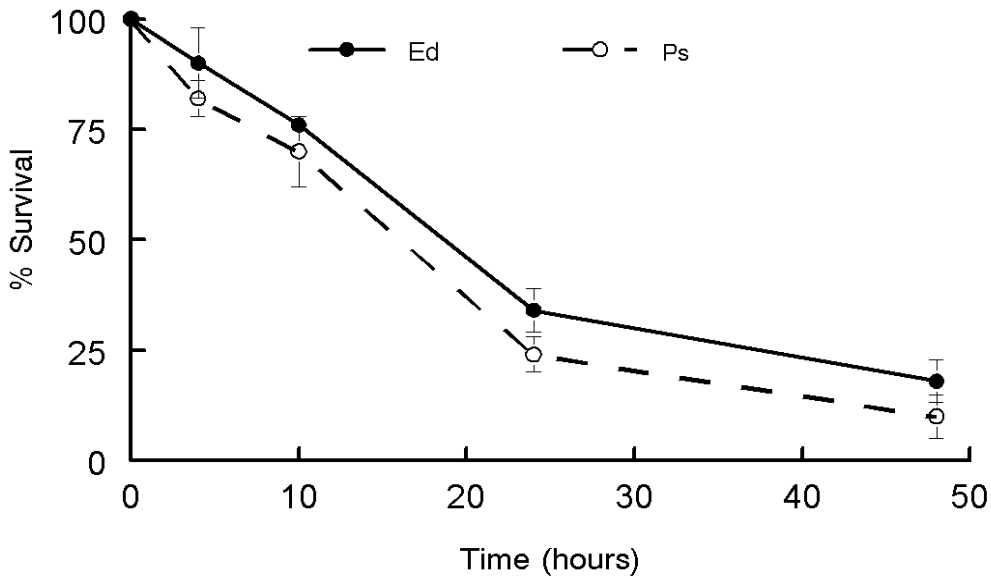

Figure 4.2: Percent survival of *P. simpsoni* and *E. depressus* (± SE) exposed to blue crab predation, measured over 5 time intervals.

Discussion

The quality and quantity of refugia are important factors regulating marine crustacean populations. Refuge limitation acting on a specific size class may create a population bottleneck that limits overall production through mortality, migration or stunting of the size class affected (Caddy and Stamatopoulos, 1990; Beck, 1995). Competition for shelter occupancy is common in xanthid crabs. Gibbs (1994) documented that juvenile *M. adina* exhibited intraspecific competition as well as interspecific competition with *E. depressus* for shelters in coastal Alabama waters. In the laboratory portion of that study, *M. adina* out competed similar-sized *E. depressus* for shelters. In my laboratory experiments, *E. depressus* was clearly dominant over *P. simpsoni* in occupying the shelters at both salinity levels. Brown et al. (2005) also suggested a dominance hierarchy with *M. adina* being the most dominant, *E. depressus* being intermediate and *P. simpsoni* being the least aggressive. My results support this suggested dominance hierarchy.

For both species, crabs occupied fewer shelters at the lower salinity. In an earlier study (Hulathduwa et al., Chapter 3, submitted) I found that both these crab species exhibited lowered scope for growth (energy used for growth and reproduction) below 17.5 PSU. Both species may be less active at lower salinities and this may reduce their chances of finding and occupying the shelters. However, *P. simpsoni* exhibited a relatively larger decrease in shelter occupancy at lower salinity. May (1974) reported that *E. depressus* was common at lower salinities at more estuarine

sites while *P. simpsoni* was most common at high salinity coastal sites in Mobile Bay. In an earlier study (Hulathduwa et al., Chapter 3, submitted) it was found that *E. depressus*is more tolerant of salinities lower than 10 PSU, perhaps allowing *E. depressus* to be more active at lower salinity, enabling it to search for and occupy more shelters than *P. simpsoni*.

In general, the most important effect of structural complexity in marine habitats is assumed to be an increase in the amount of refuge from predators (Hixon and Beets, 1993). However, shelters could also deter fouling and reduce parasitism or help enhance resource defense of mates or food. Shervette et al. (2004) studied competition for shelter among xanthid crabs in relation to predation pressure from the oyster toad fish *Opsanus beta*. They found that *M. adina* may be limited by shelter availability because of its vulnerability to predation. Beck (1995), also documented that in his study area (St. Joseph Bay, Florida) the habitat appears to provide ample shelters for small *M. adina*, but large crabs find few areas with appropriate structural relief in which to shelter or initiate burrows.

C. sapidus captures fast moving soft-bodied prey, as well as hard-shelled, bivalve prey (Hughes and Seed, 1995). Prey selection can be the consequence of active decisions throughout the foraging bout (Hughes and Seed, 1981), and thus may reflect prey profitability or could be passive, resulting from a greater chance of encountering items with a larger surface area and/or reduced handling times (Seed, 1993). Previous studies of prey selection by *C. sapidus* on fiddler crabs, *Uca pugilator*, documented that movement, larger size, and brighter coloration increased prey risk (Hughes and Seed, 1995).

Mascaro et al., (2003) examined size-selective foraging of *C. sapidus* on the shrimp *Litopenaeus setiferus* and also found larger shrimp were more predation-prone by large (90-110 mm carapace width) blue crabs. They also found that the foraging behavior of 30-50 mm carapace width *C. sapidus* was passive, and did not reflect prey profitability.

My results indicate survival of both *E. depressus* and *P. simpsoni* was significantly affected by time, as they were preyed upon by the blue crab. Although the mechanism of prey selection was not identified in this experiment, crabs of similar size (carapace width < 3.5 mm) were used, ruling out any size effect. *E. depressus* exhibited a higher survival after 48 hours. The ability of *E. depressus* to better defend shelters may play a key role in leading to better survival.

Co-existence of crab species is generally associated with differences in size (Turra and Leite, 2002), micro-habitat use (Gherardi and Nardone, 1997), activity rhythms (Barnes, 2002), or tolerance of desiccation (Bertness, 1981). Differences in vertical distribution, feeding niches and salinity tolerances may limit competition between the two co-occurring mud crab species *E. depressus* and *P. simpsoni* (May, 1974: Meyer, 1994). *E. depressus* is known to be associated with the upper portions of the oyster reefs, and Meyer (1994) suggested their smaller size facilitates hiding in crevices among oysters. McDonald (1982) suggested that *E. depressus* is more omnivorous, and exhibits a more "*r*-selected" life-history strategy allowing niche partitioning. My results indicate that *E. depressus*'s ability to tolerate lower salinities, and its dominance in resource holding potential, may lessen predation risk and allow colonization of more estuarine sites.

Chapter 5
Summary and Conclusions

This study indicates that hydrocarbon pollutants, salinity, and competition may influence the diversity, abundance and distribution of organisms that colonize oyster reefs. The trays were readily colonized by invertebrates and fish. Meyer and Townsend (2000) also reported rapid colonization of created reefs. The results of the first chapter suggest that the exposure to hydrocarbons reduces the diversity and abundance of commensal organisms, although the effect was not as prominent as that of salinity. Only two species (the toad fish *O. beta* and mud snail *N. acutus*) consistently had reduced abundances in oil-treated cultch. Lee et al. (1981) also reported that dosing of a *Spartina* marsh with hydrocarbons resulted in no decrease in oyster, mussel and fiddler crabs but that periwinkle densities did decline. In this study, one of the most-severely impacted species was the small detritivorous mollusc, *Nassarius*. Most arthropods and fish were either not affected by hydrocarbons, or were mobile enough to avoid contaminants. Oyster reefs and associated fauna may be pre-adapted to hydrocarbon spills due to long-term oil production along the Louisiana coast line (Carman et al., 2000; McCoy and Brown, 1998).

Salinity was the key factor determining the colonization of oyster reefs as in other studies (Wells, 1961). This study shows that *E. depressus* is more tolerant of low salinities than *P. simpsoni.* Salinity significantly affected the food consumption, energy absorption, energy lost as respiration and scope for growth of both species. However, having a low but a positive value for scope for growth indicates that both species are capable of surviving and functioning at salinities as low as 7.5 PSU. The peak scope

for growth occurs at 17.5 PSU for both species. Results of this study also indicate that both species are hyper-osmoregulators and that their osmoregulatory ability does not differ. Although *E. depressus* was found to be more tolerant of low salinities than *P. simpsoni*, the physiological responses studied in the third chapter did not significantly differ between the two species, and cannot therefore explain the different tolerances.

McDonald (1982), documented that a combination of trophic and microhabitat factors could explain the co-occurrence of *P. herbstii* and *E. depressus* in South Carolina. Competition for shelter occupancy is common in xanthid crabs and the availability of refuges influences the density and size structure of many marine crustacean populations (Caddy and Stamatopoulas, 1990; Beck, 1997; Brown et al., 2005). The results of the fourth chapter demonstrate that *E. depressus* is dominant over *P. simpsoni* in occupying the shelters at both normal (ambient) and low salinity levels. Both crab species exhibited lowered shelter occupancy at low salinity. However, *P.simpsoni* exhibited a relatively larger decrease in shelter occupancy at lower salinity perhaps because of its lower tolerance to salinity. This lower tolerance to salinity and lower shelter occupancy may increase predation risk of *P.simpsoni*, and explain its more stenohaline distribution. Although *C. sapidus* preyed upon both mud crabs, *E. depressus* exhibited higher survival after 48 hours. *E. depressus* may have a better chance of avoiding the predator based on its ability to better defend shelters. Higher tolerance to low salinity coupled with dominance in resource holding potential thus may facilitate *E. depressus*'s ability to colonize more estuarine sites.

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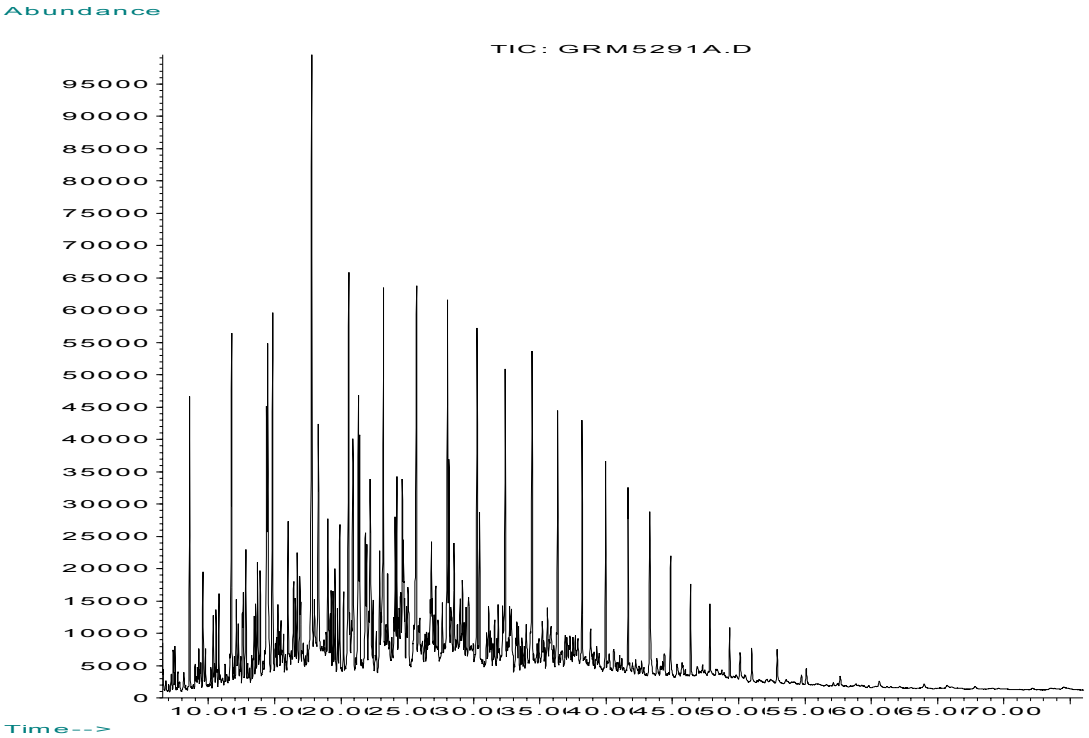
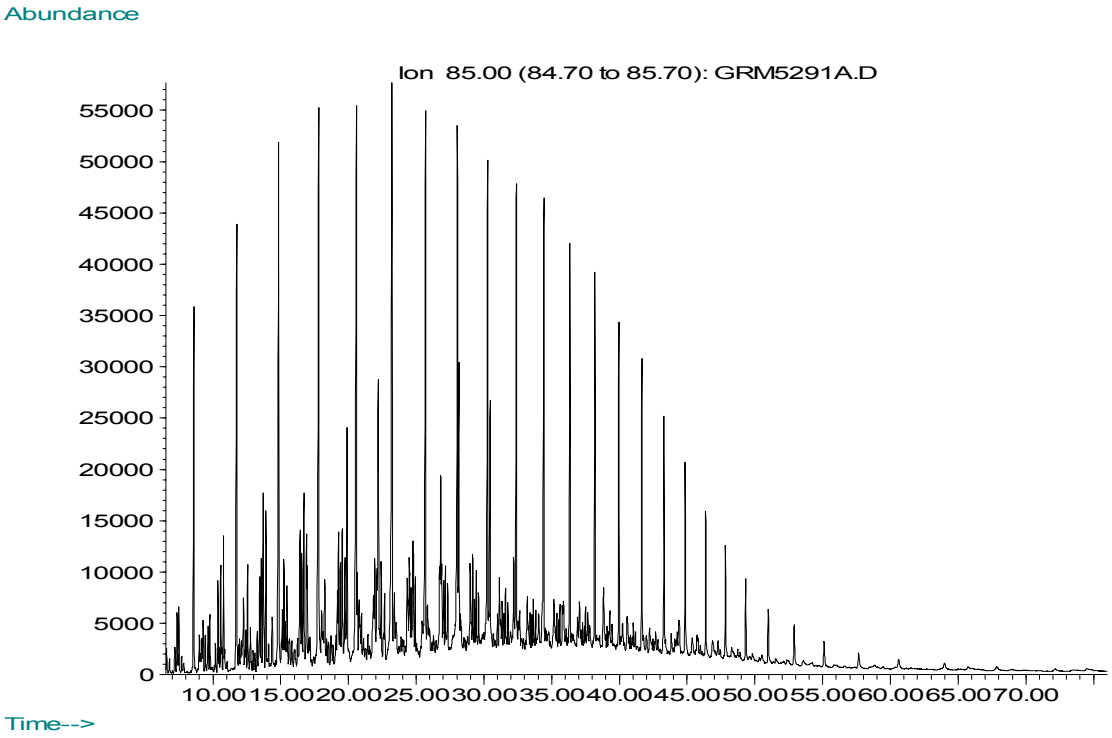
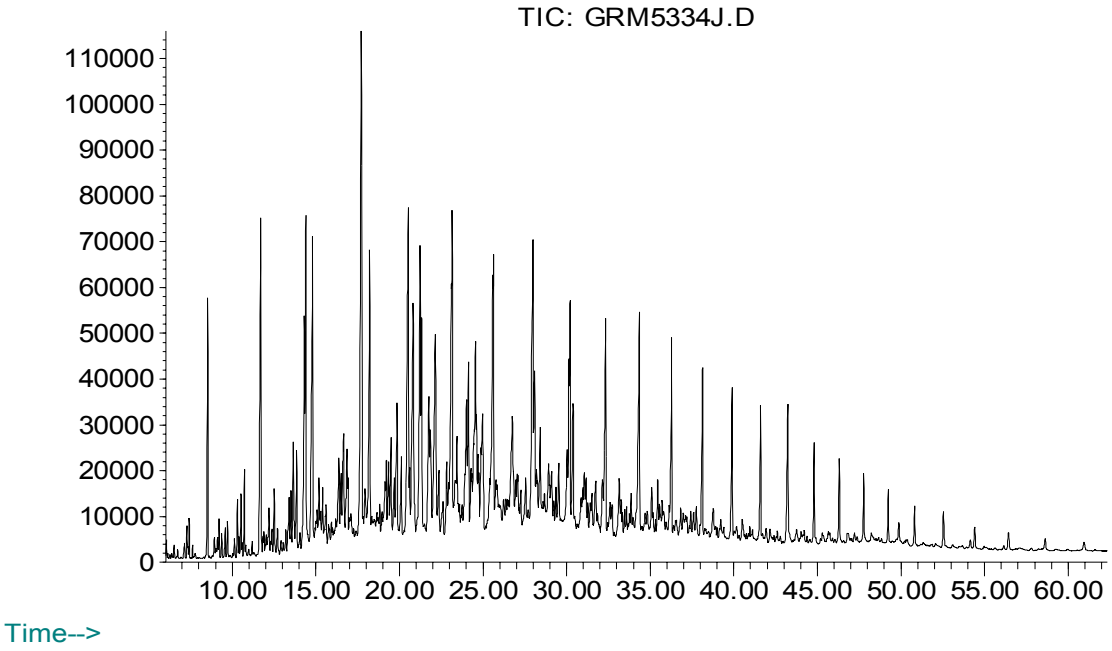
Appendix: Chromatograms of Gc/Ms Analysis



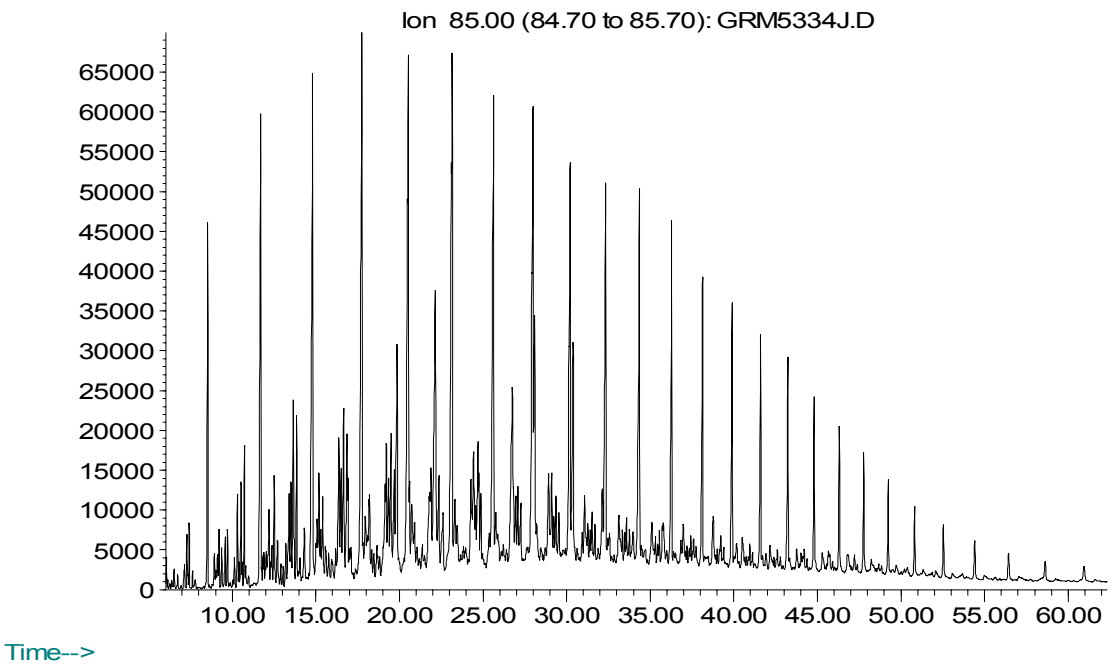
Reference Oil -- Total Ion Chromatogram



Reference oil -- Normal Alkanes



Initial Oil Shell -- Total Ion Chromatogram



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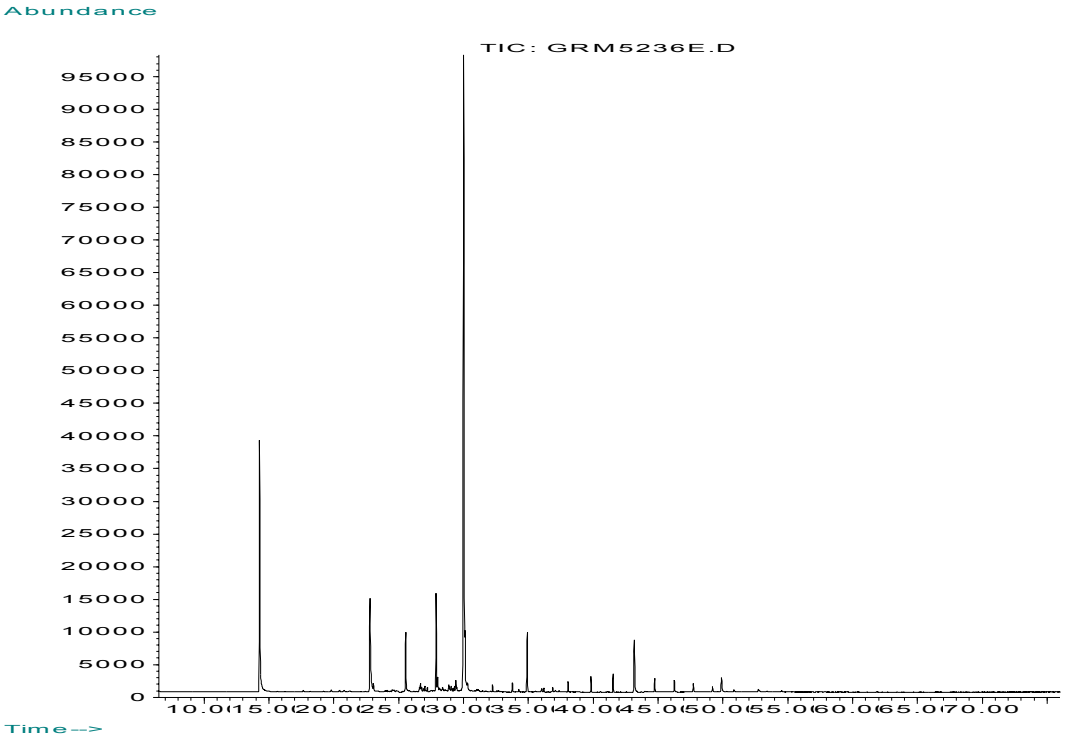
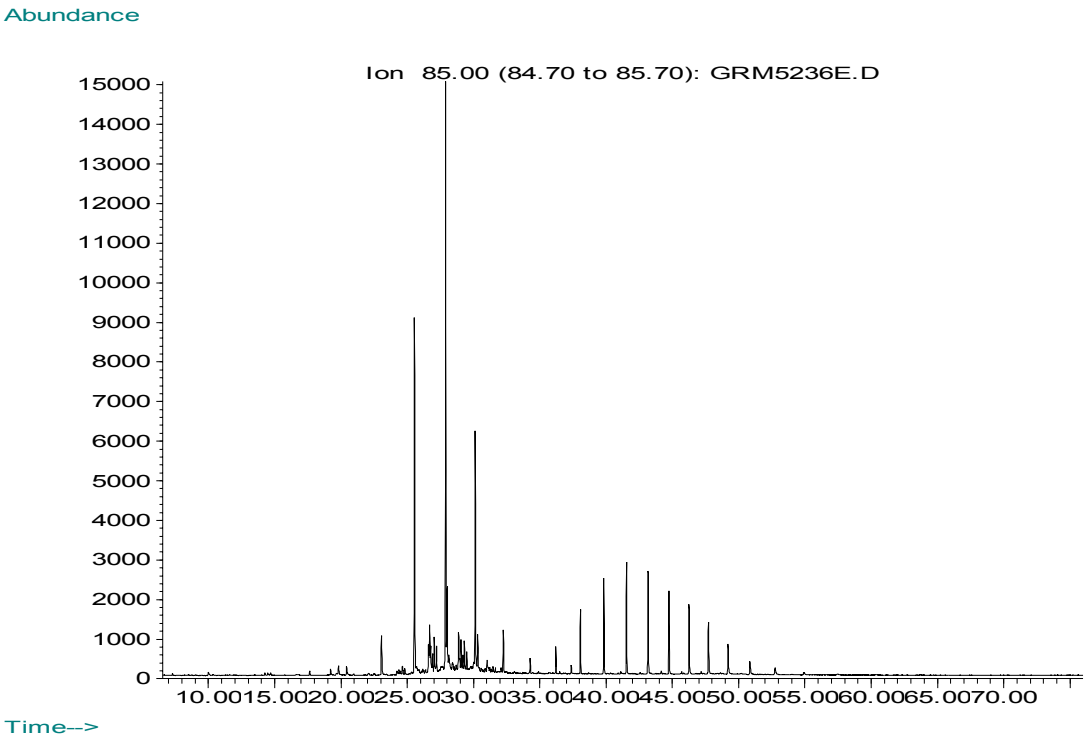


Sub Control Shell w/o Mud -- Total Ion Chromatogram



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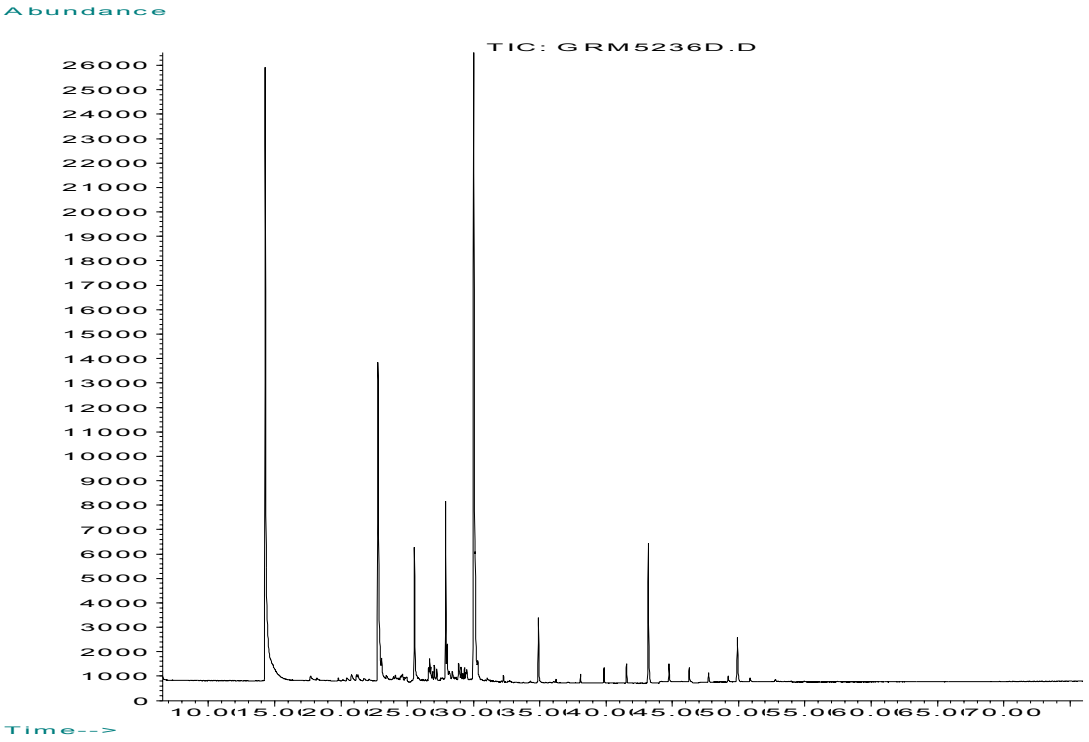
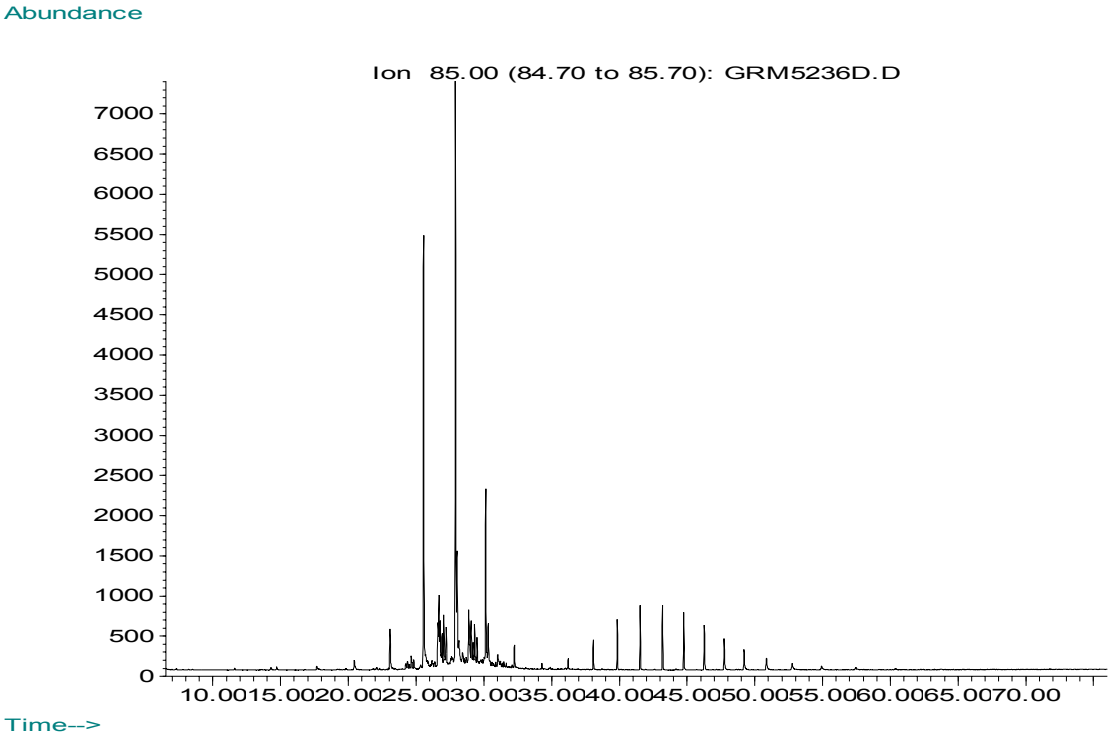


Sub Control Shell with Mud -- Total Ion Chromatogram



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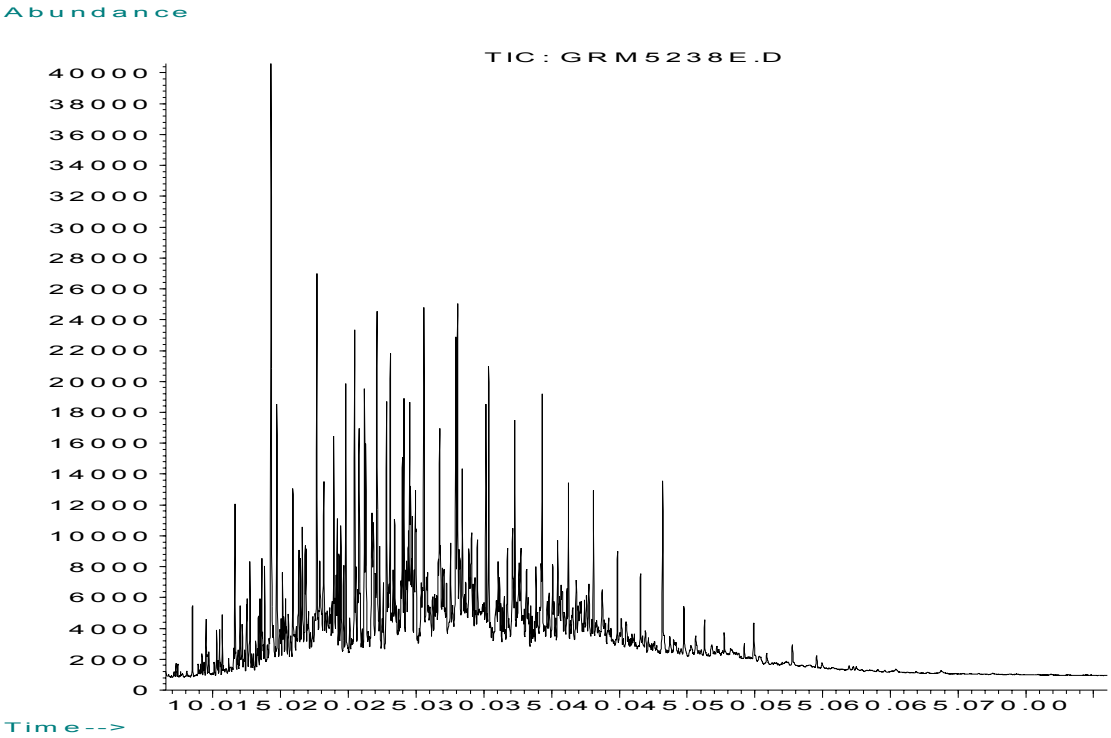

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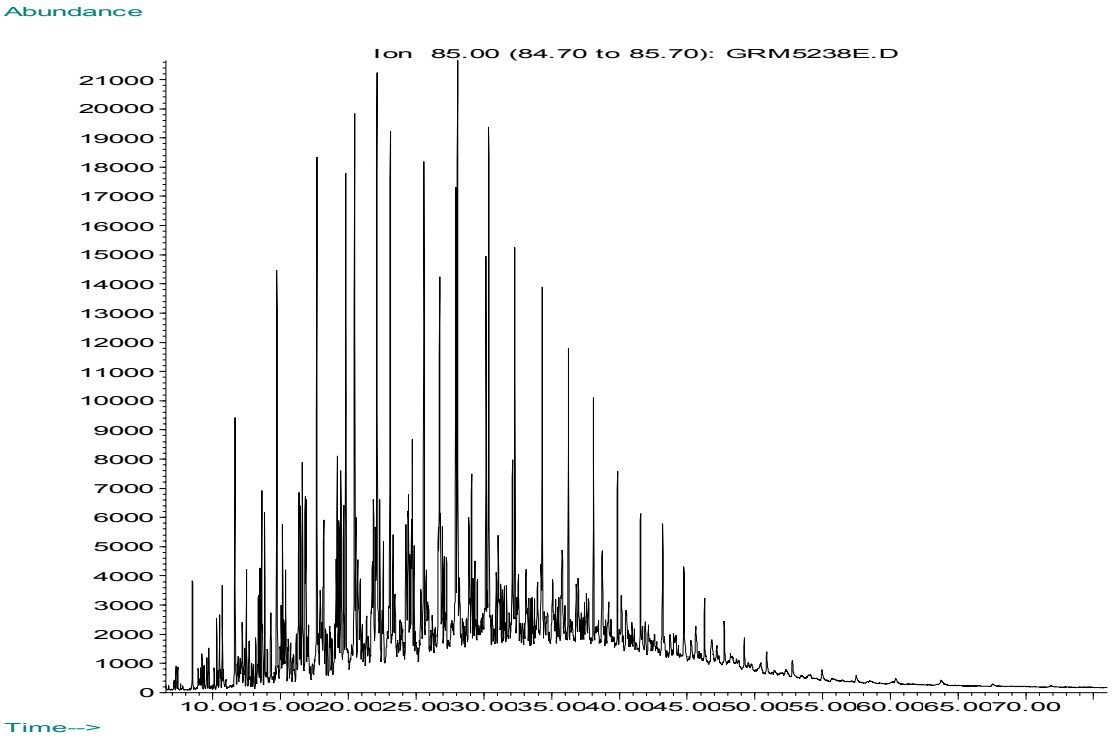
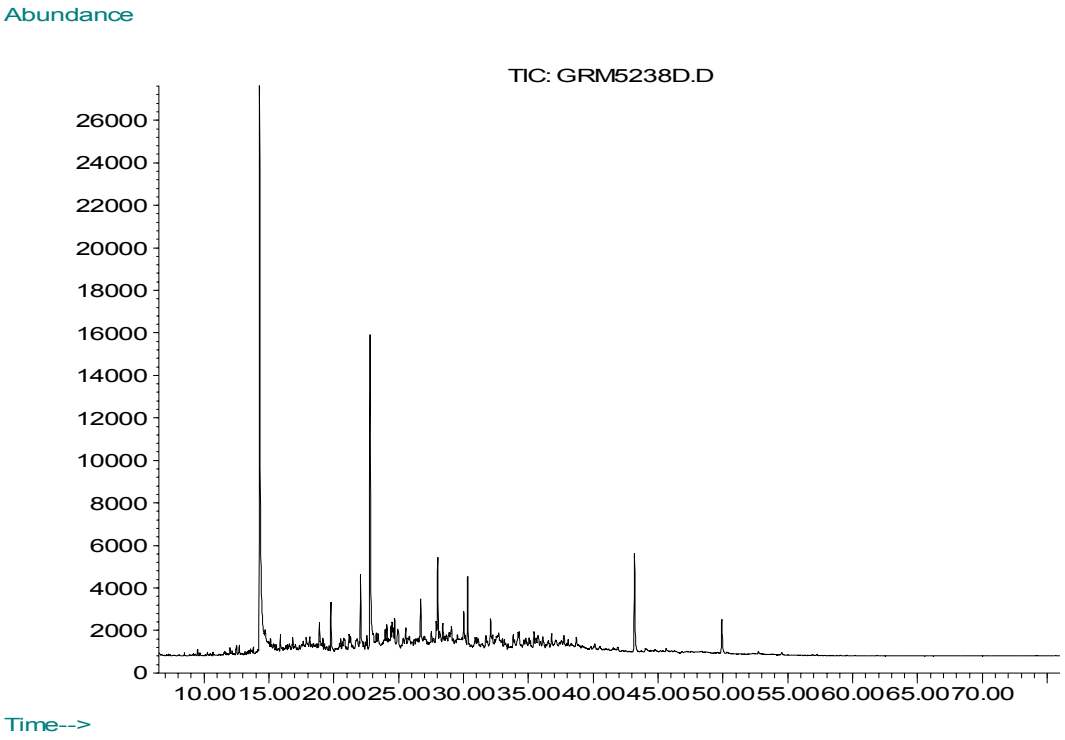
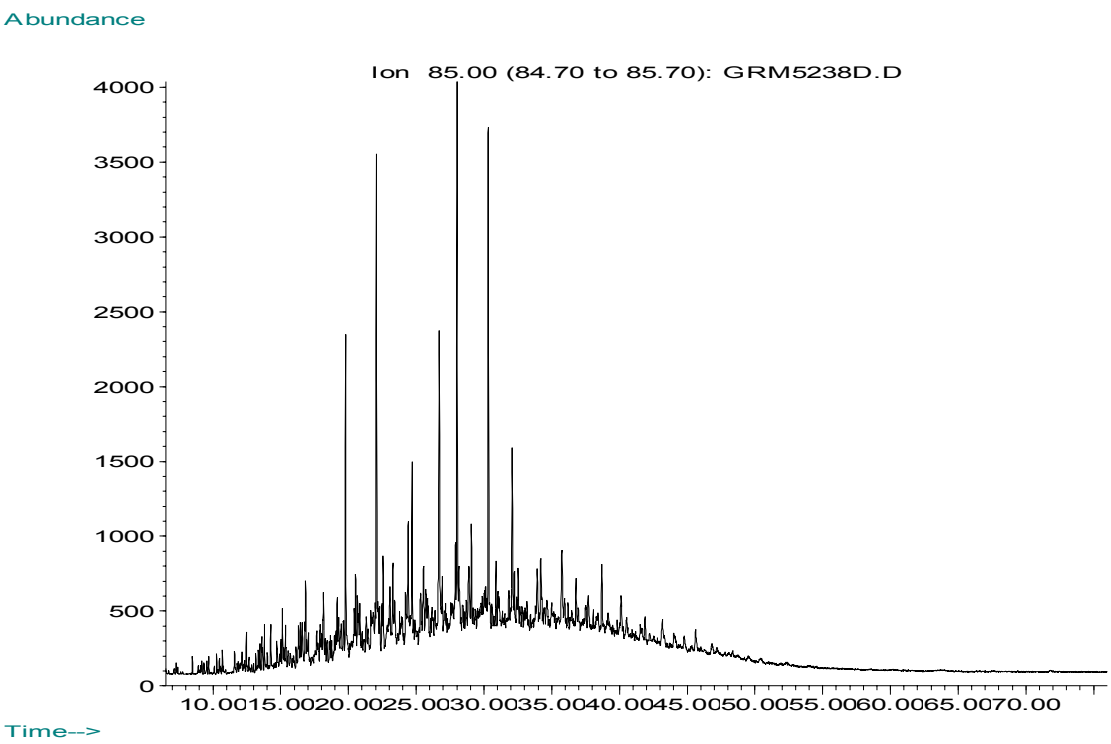


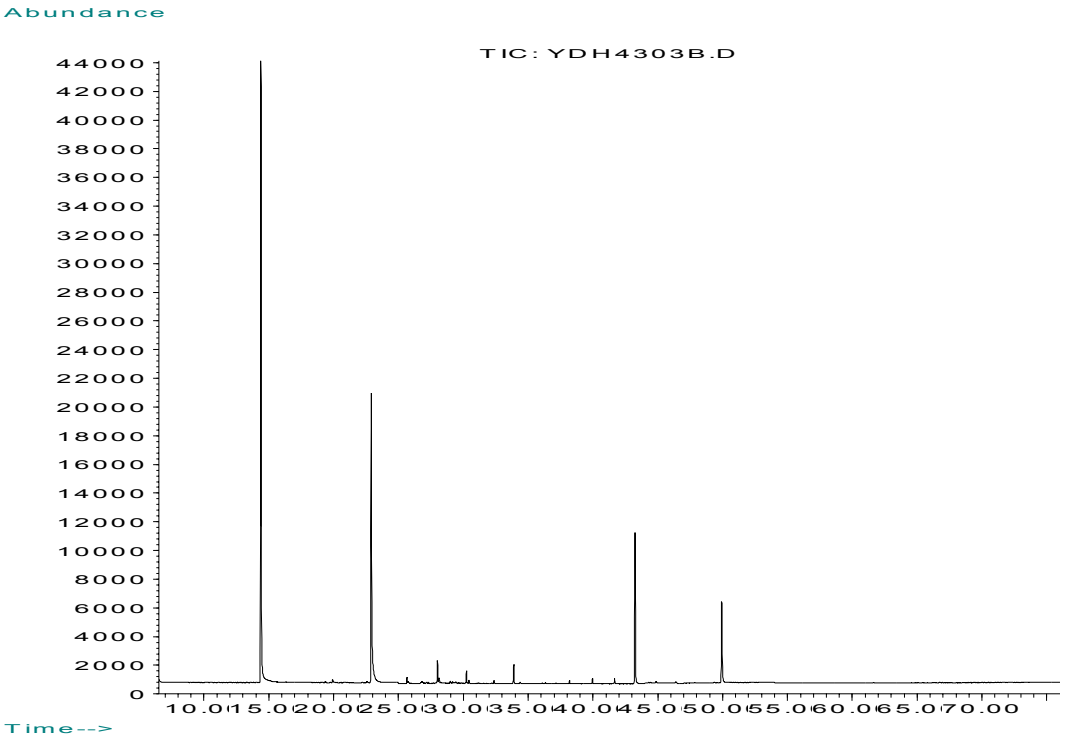
Sub Oil Shell w/o Mud – Normal Alkanes



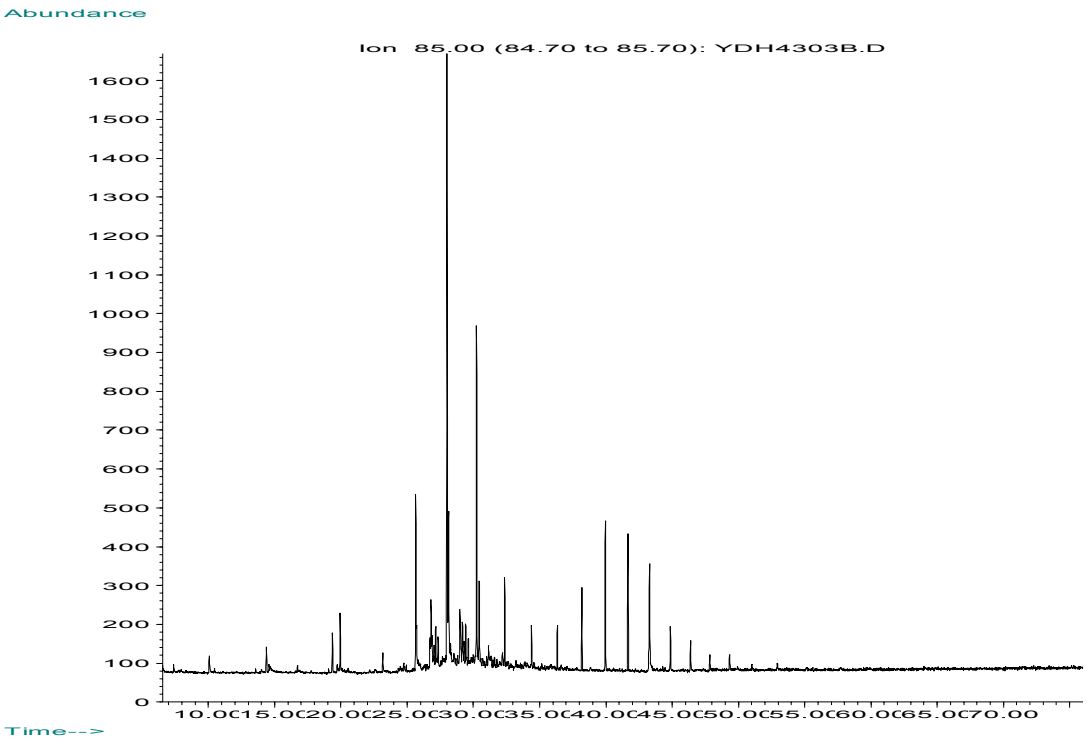
Sub Oil Shell with Mud -- Total Ion Chromatogram



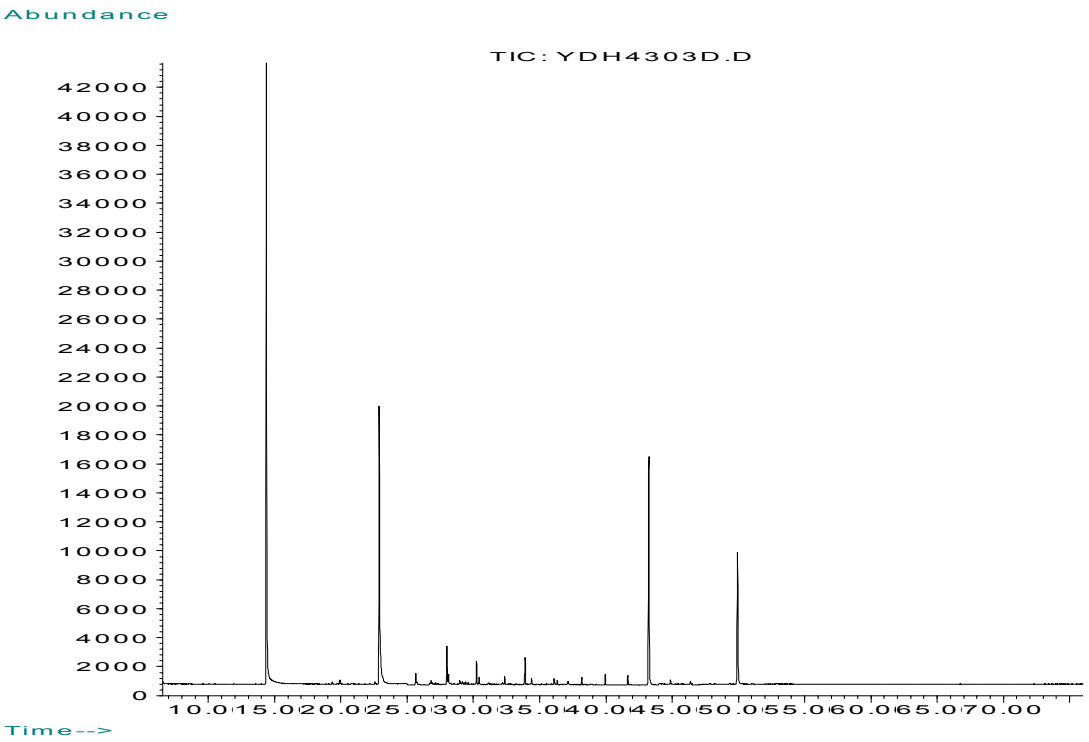
Sub Oil Shell with Mud – Normal Alkanes



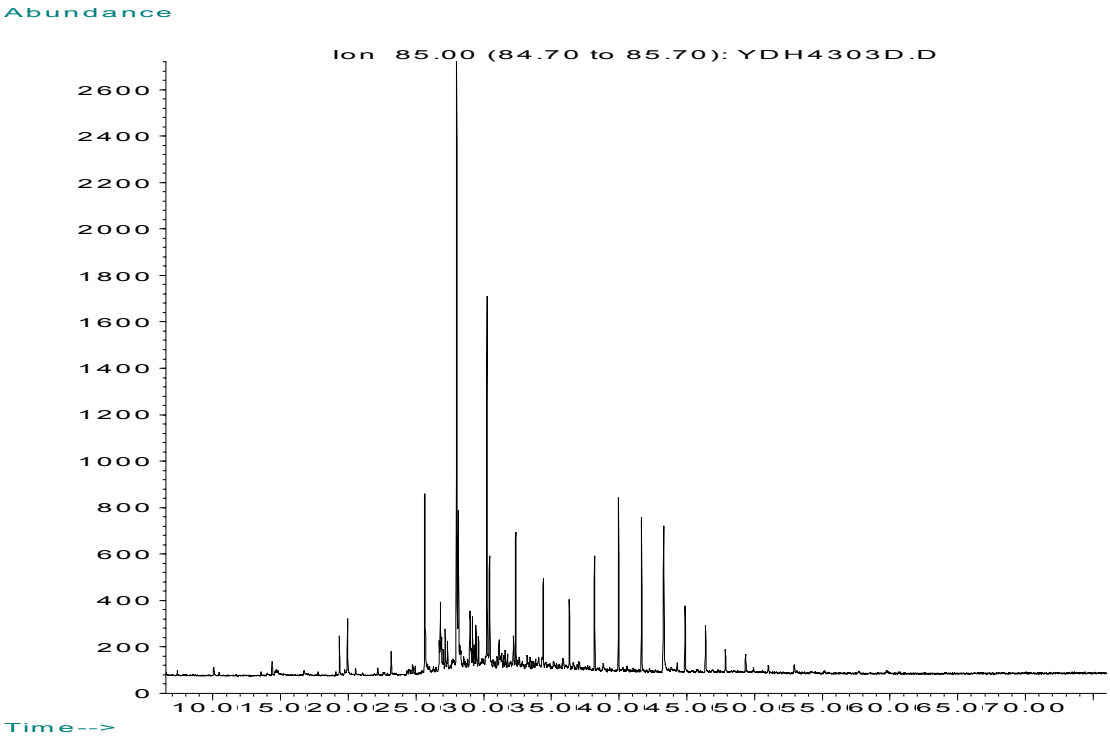
Control Inter w/o Mud – Total Ion Chromatogram



Control Inter w/o Mud – Normal Alkanes



Control Inter with Mud – Total Ion Chromatogram



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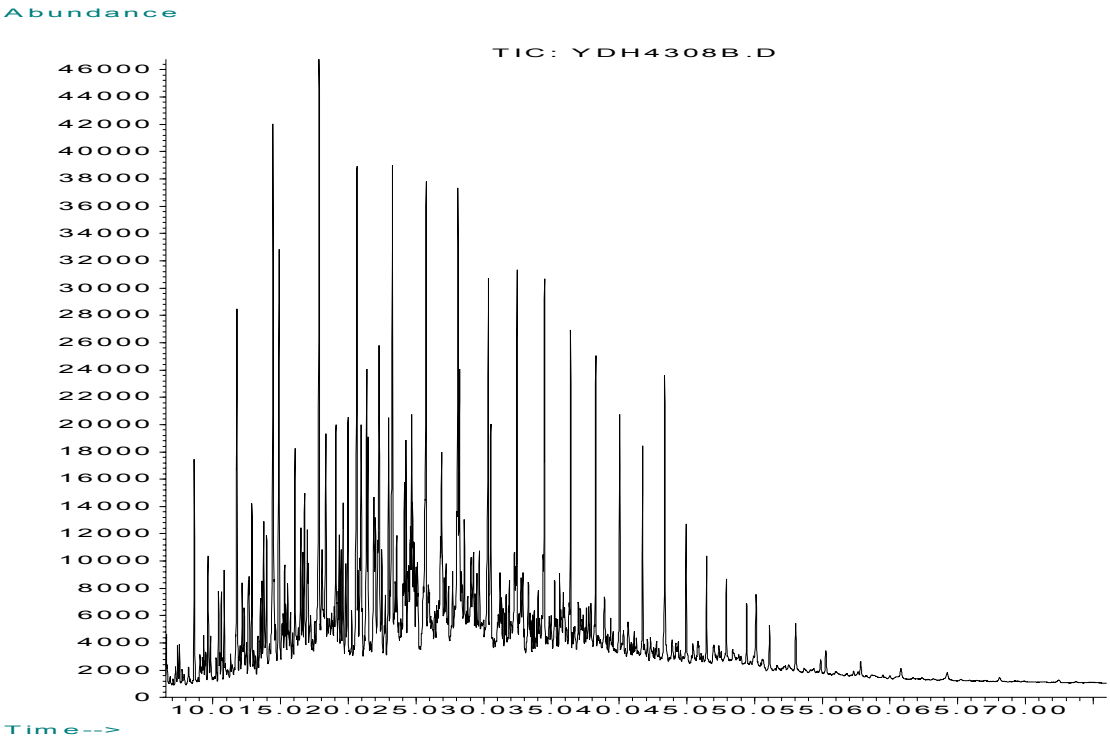
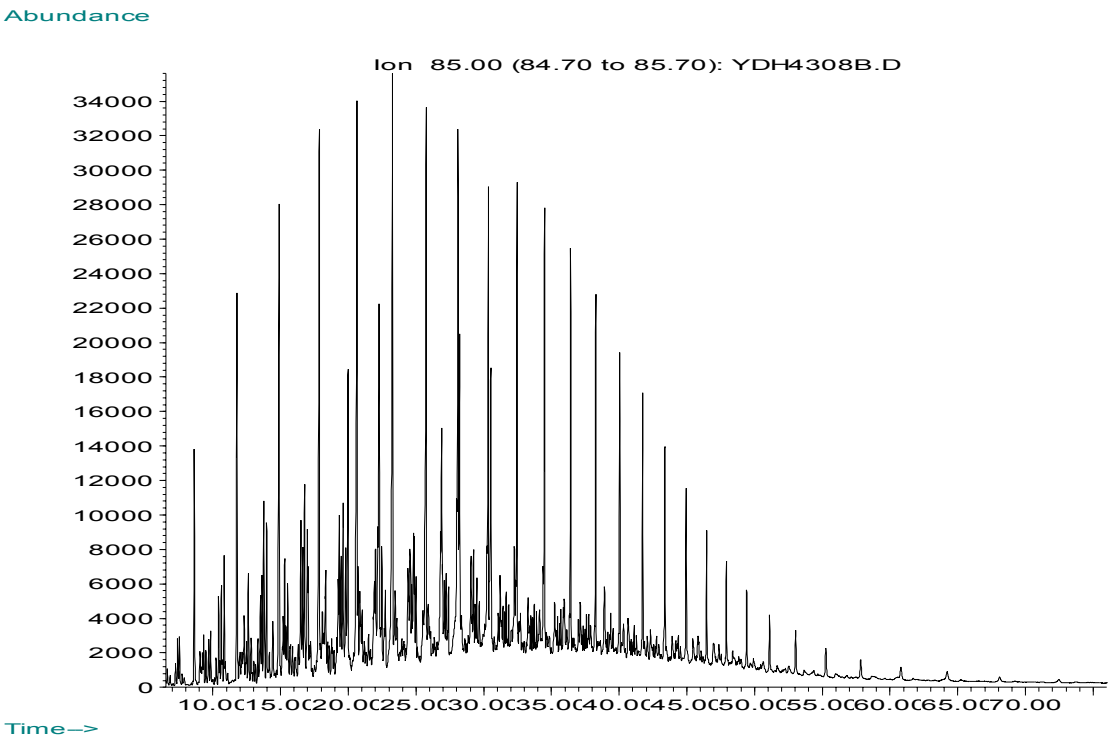


Oil Inter w/o Mud – Total Ion Chromatogram



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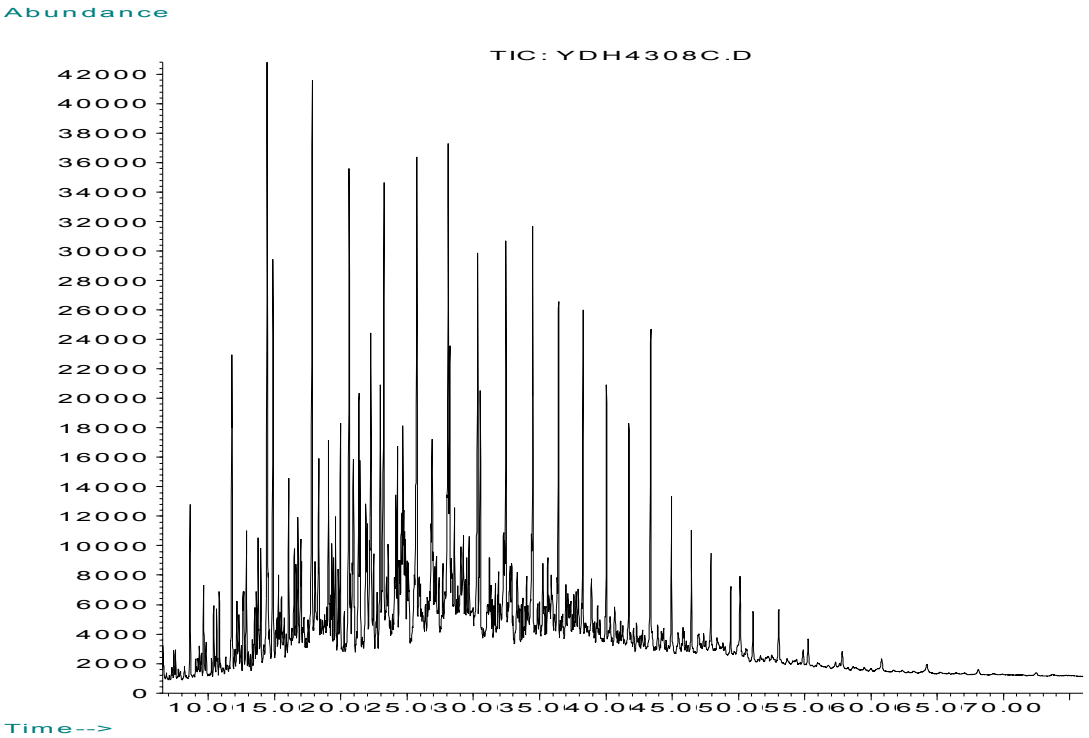

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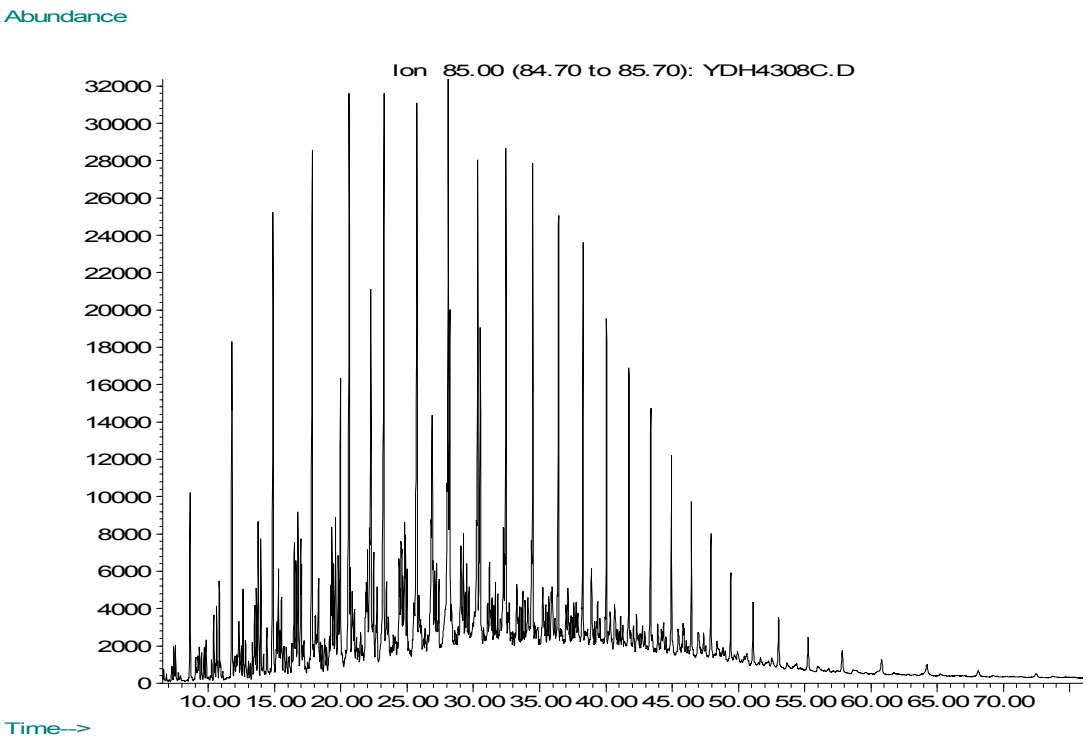

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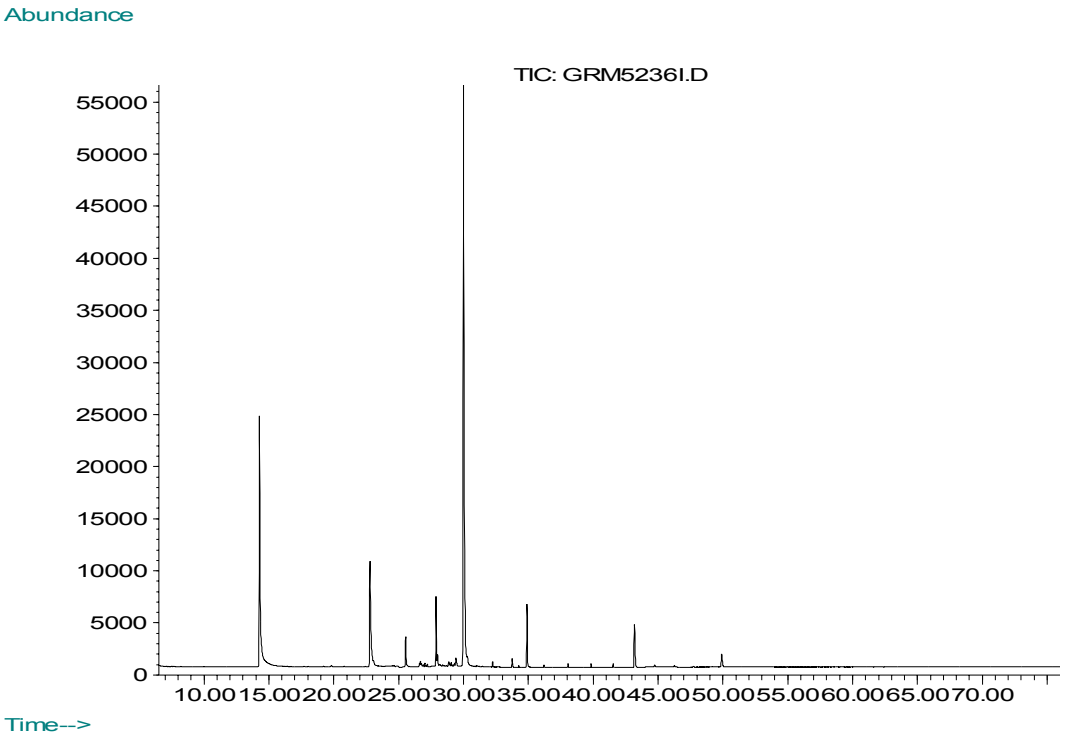

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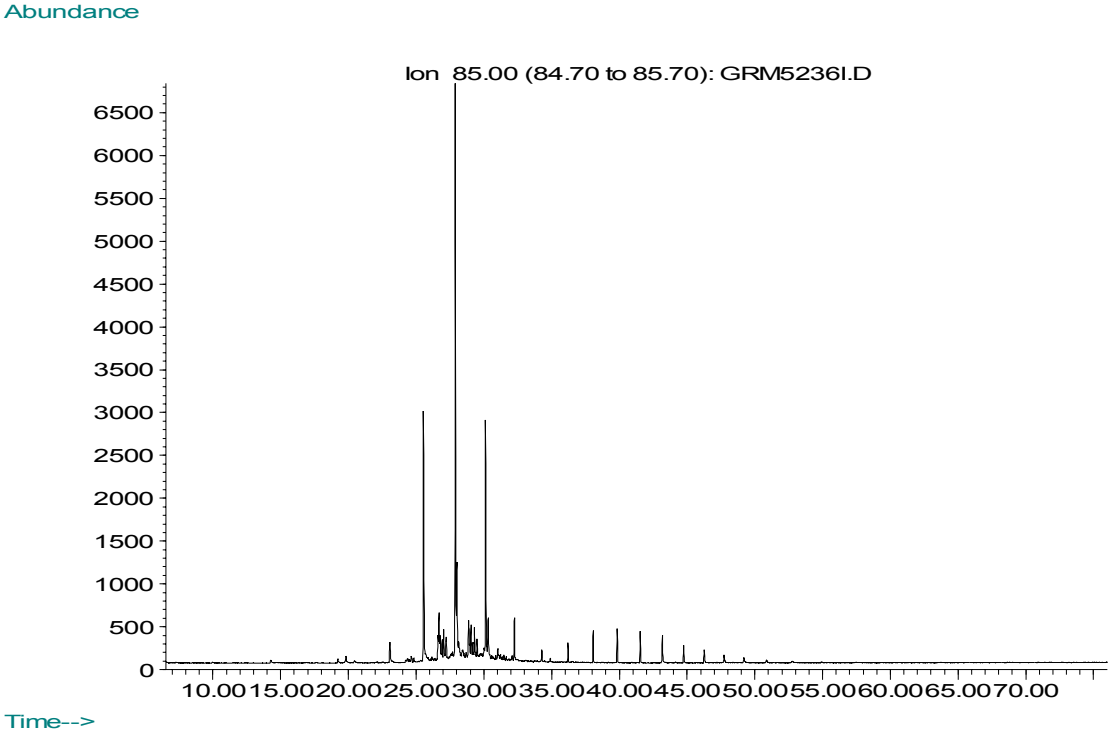
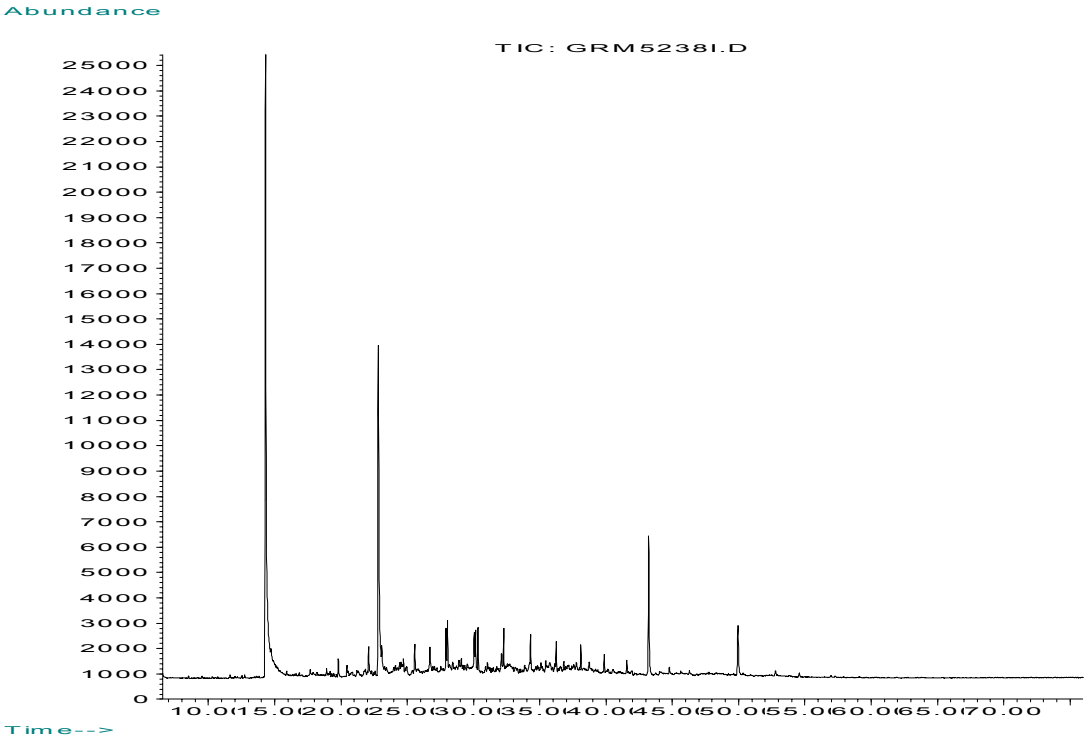


Estuarine Control Shell w/o Mud – Normal Alkanes



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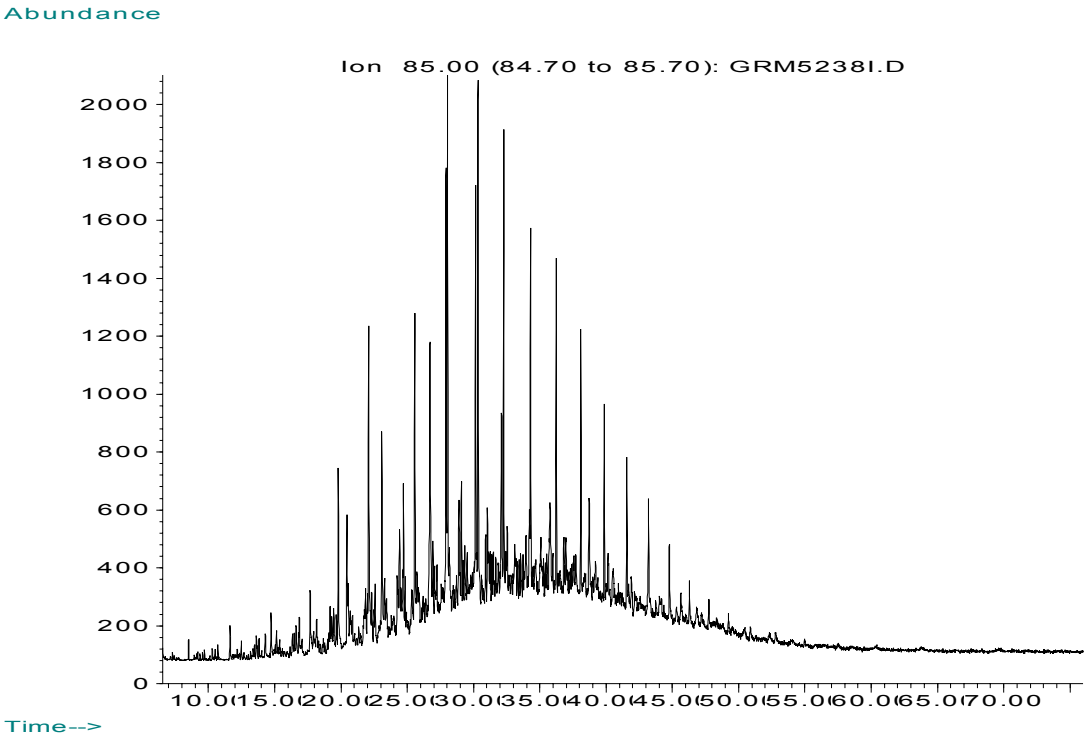

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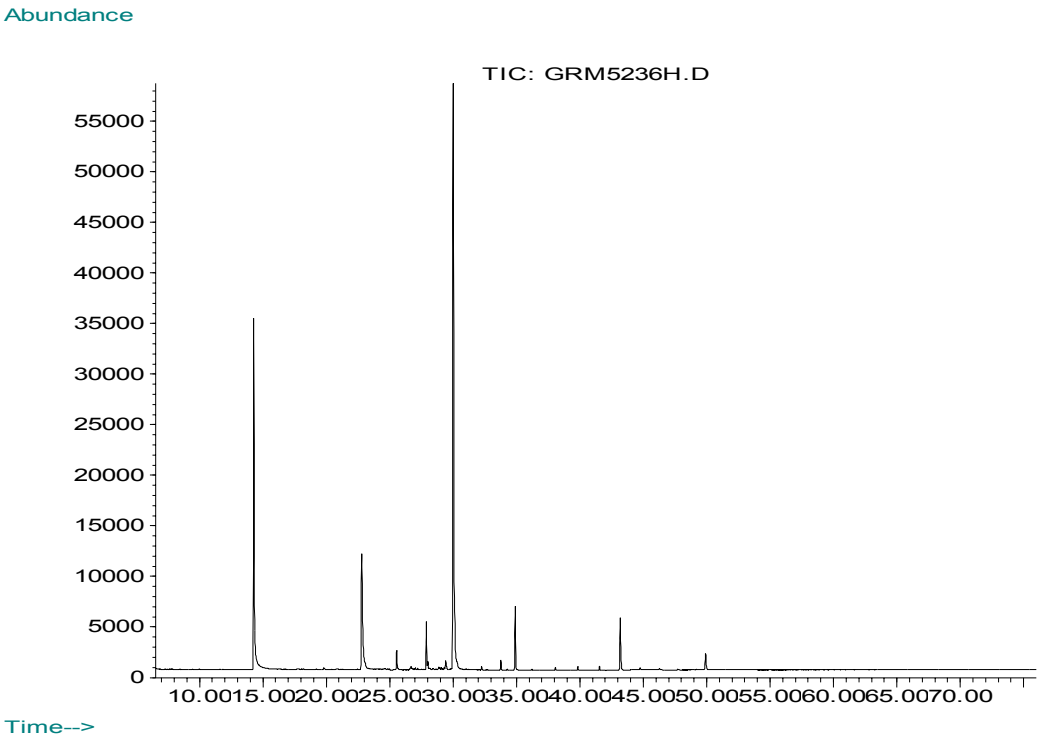

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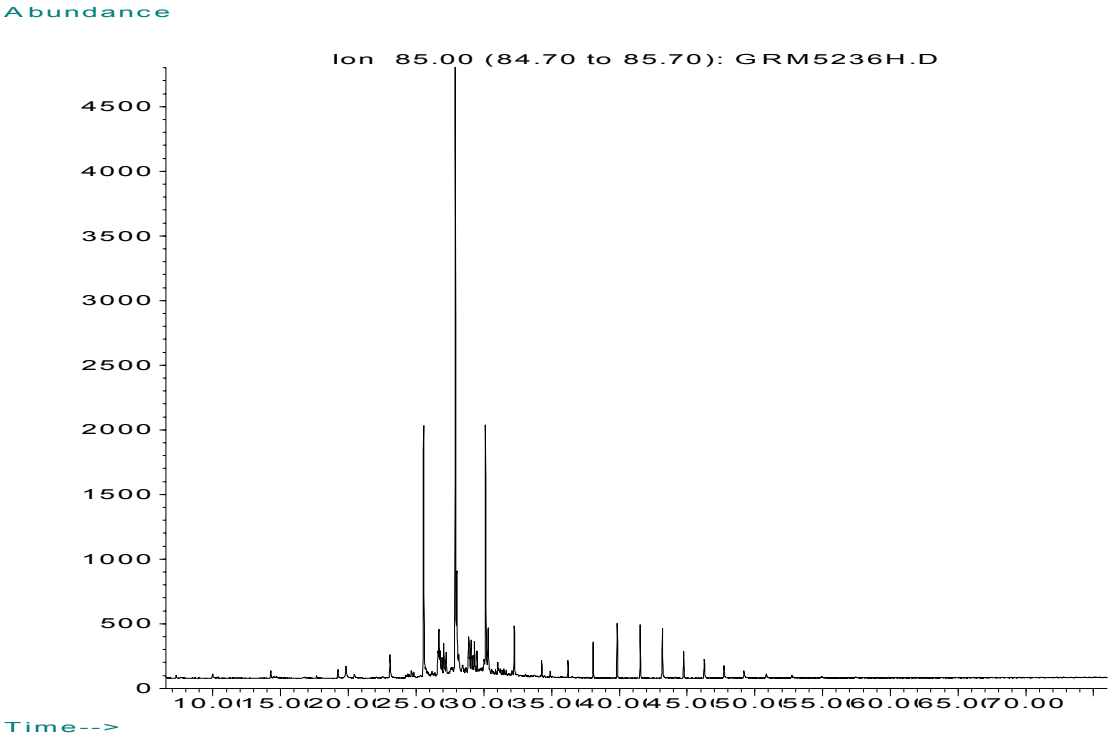
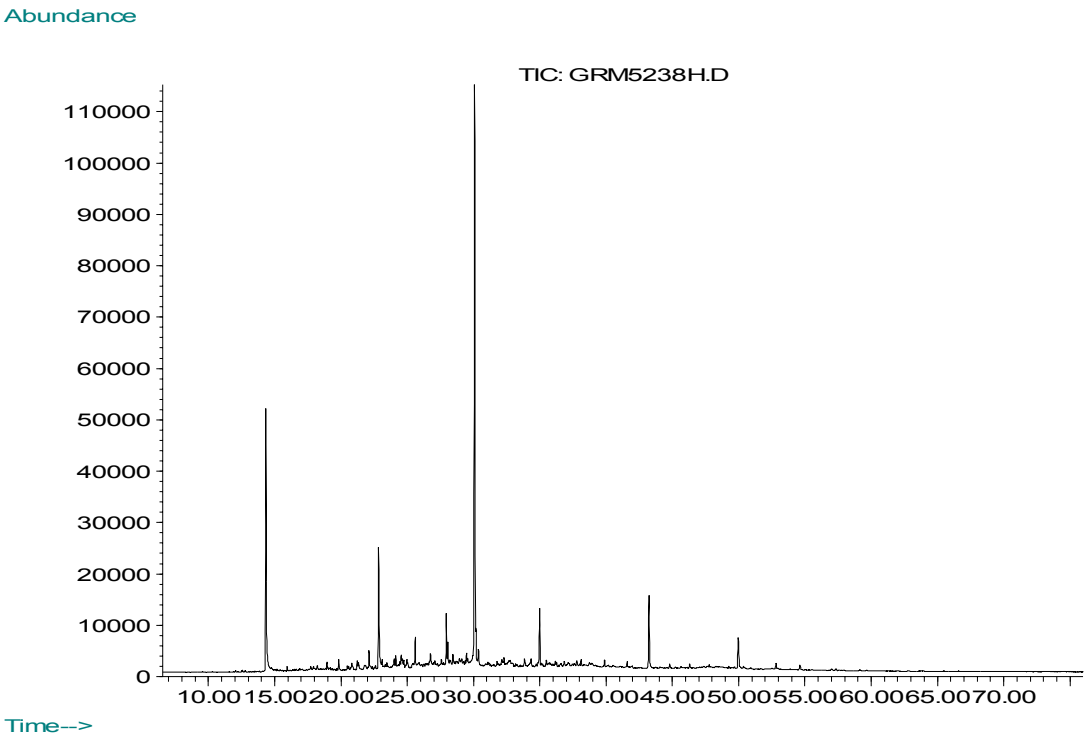
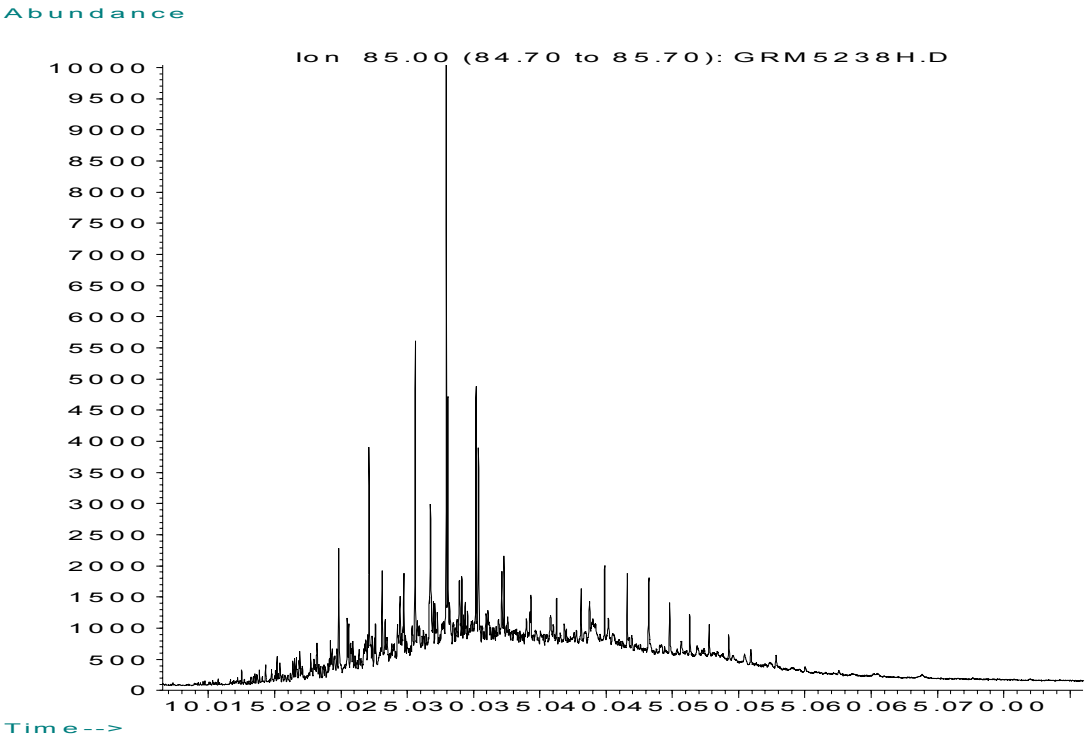


Estuarine Control Shell with Mud – Normal Alkanes



Estuarine Oil Shell with Mud – Total Ion Chromatogram



Estuarine Oil Shell with Mud – Normal alkanes

Vita

The author, Yasoma Dhammika Hulathduwa, is the daughter of Hulathduwage Don Wijedasa and Malini Chandrasena. She was born on March 8th, 1972 in Colombo Sri Lanka. She is married to Cecil Yapa and is the mother of Yasith Yapa.

She attended the University of Sri Jayewardenepura and graduated in September 1999, with a bachelor of science degree in Zoology. She received Professor Winston Rathanayake memorial award for being the best Zoology student in the class of 1999. After working as a teaching assistant in the same university for one year, she then enrolled in the Ph. D. program in the department of Biological Sciences, Louisiana State University in fall of 2000. She was awarded William Gates award for teaching excellence from the Department of Biological Sciences, Louisiana State University in spring of 2005. Her dissertation was completed in spring of 2006, under the guidance of Professor Kenneth M. Brown. Research publications based on her dissertation have been submitted to *Marine Environmental Research* and *Marine Biology*. She has also presented results of her research at national meetings, including at the 32nd annual Benthic Ecology Meeting in spring of 2003.